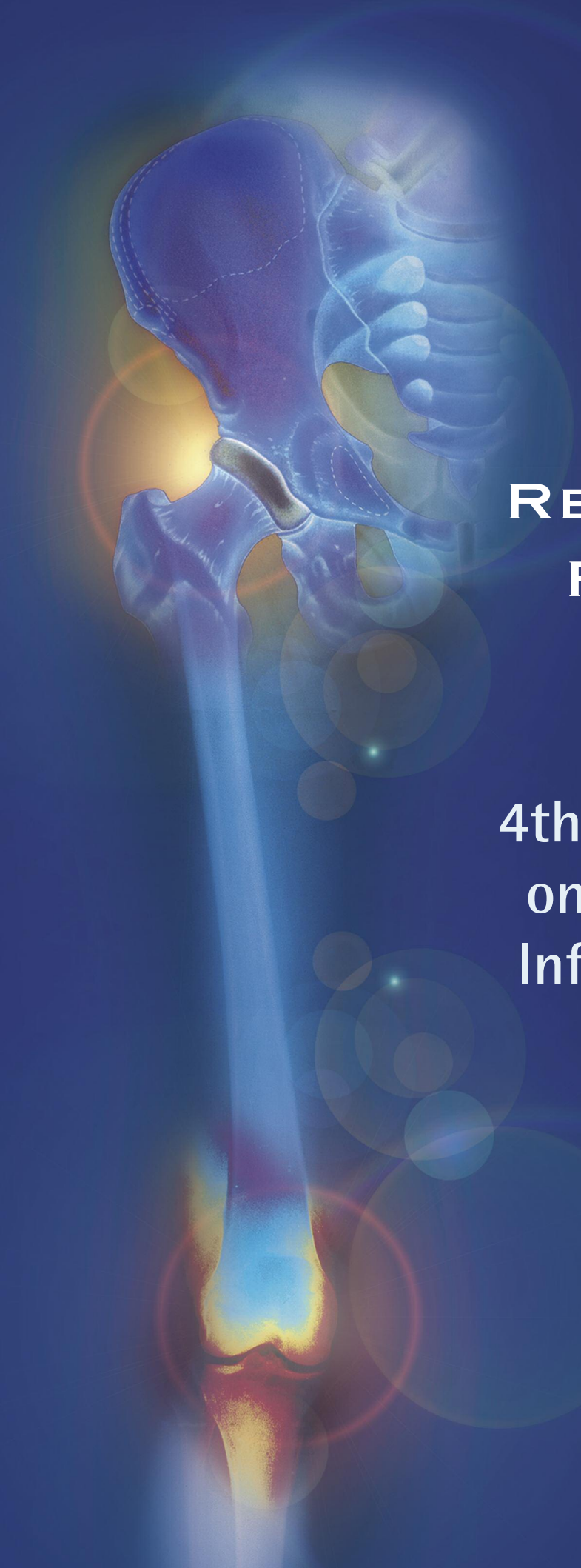

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ABSTRACTS

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POSTER PRESENTATIONS

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INVITED LECTURES

I1 – Inflammasome Activation: From Basic Concepts to Therapeutic Target

Netea M.G.
Dept. of Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Interleukin-1β (IL-1β) and IL-18 are important proinflammatory cytokines that on the one hand activate monocytes, macropages and neutrophils, and on the other hand induce Th1 and Th17 adaptive cellular responses. They are secreted as inactive precursors, and their processing depends on cleavage by several protein platforms called the inflammasomes.

Inflammasome activation differs in various cell types, and knock-out mice defective in either caspase-1 or inflammasome components display a changed susceptibility to inflammatory conditions. Activation of the inflammasome has been shown to be induced by several danger signals released during inflammation and autoimmune processes, and the involvement of inflammasome in the pathogenesis of autoimmune and autoinflammatory diseases is intensely studied. An overview of the current concepts in the field will be presented, followed by recent insights in the role of the inflamamsome activation for the pathogenesis of several important autoimmune and autoinflammatory diseases.

I2 – Regulation of Adaptive Immunity by Innate Immune Receptors

Reis e Sousa C.
Immunobiology Laboratory, Cancer Research UK, London Research Institute, London, UK

Direct sensing of pathogen components is a major trigger of dendritic cell (DC) activation, leading to adaptive immunity. We have been studying multiple pattern-recognition pathways that mediate DC activation. One pathway for sensing infection by RNA viruses involves recognition of viral genomes or virally-infected cells in endosomal compartments and utilises members of the toll-like receptor (TLRs) family, including TLR9, 7, or 3. Viral genomes can additionally be recognised in the cytosol by DExD/H-box helicases such as RIG-I, which are activated by RNAs bearing 5' triphosphates. Finally, a distinct pathway involves cell surface and phagosomal recognition of fungi by C-type lectins, which signal via Syk kinase. Notably, some of these pathways are involved not only in direct sensing of pathogens but also in the recognition of self alterations that might accompany infection, such as induction of cell death.

These studies help build a global picture of the receptors and signalling pathways that regulate DC activation and have applications in immunotherapy of cancer and infectious diseases.

I3 – TLR 2 and 4 Play a Critical Role in Autoantibody Production and Glomerulonephritis in Lpr Mutation-Induced Mouse Lupus

Musette P.
INSERM, U905, Rouen, France & University of Rouen, IFRMP, Institute for Biomedical Research, Rouen, France

Systemic lupus erythematosus (SLE) is a non organ specific autoimmune disease characterized by the production of pathogenic autoantibodies directed against nuclear antigens and immune complex deposits in kidneys. Environmental factors have been thought to play a role in the onset of the disease in particular through Toll-like receptors (TLR). In this respect we recently demonstrated the role of TLR9 in the production of anti-nucleosome autoantibodies.[1,2]

The goal of this study is to determine the role of TLR2 and TLR4 in the development of spontaneous lupus disease by creating TLR2 or TLR4 deficient C57BL/6$^{lpr/lpr}$ mice. TLR2 and TLR4 deficient lupus prone mice have been generated by crossing C57/BL6-TLR2$^{-/-}$ or C57/BL6-TLR4$^{-/-}$ mice with C57/BL6$^{lpr/lpr}$ mice which develop a moderate type of lupus related to Fas deficiency. We analysed the phenotype of the disease, autoantibody production and renal injury. Statistical comparisons were performed using the Mann-Whitney U-test. TLR2 or TLR4 deficient C57BL/6$^{lpr/lpr}$ mice developed a less severe disease and few immunological altera-

tions. Indeed, glomerular IgG deposits and mesangial cell proliferation were dramatically decreased and anti-nuclear, anti-dsDNA and anti-cardiolipin autoantibody titers were significantly reduced. However, the response against nucleosome remained unaffected, indicating a role of TLR2 or TLR4 in the production of autoantibodies directed against certain SLE-related autoantigens. Interestingly, the lack of TLR4 also affected the production of cytokines involved in the development of lupus disease.

Our data show that deficiency in TLR4, and at a lesser extent in TLR2, down-regulates production of autoantibodies and attenuates the development of renal injuries and thus exerts a protective role in this strain of lupus prone mice.

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I4 – Natural and Adaptive Foxp3+ Regulatory T Cells

Lafaille J.J.
New York University School of Medicine, New York, NY, USA

Foxp3$^+$ regulatory T cells (Treg cells) are essential components of the homeostasis of the immune system. Inactivating mutations in the Foxp3 gene results in severe autoinflammatory conditions, IPEX in humans and scurfy in mice. These conditions are lethal if left untreated, indicating the powerful downmodulatory effect of Treg cells.

Naturally-occurring Foxp3$^+$ T reg cells (nTreg cells) are generated in the thymus. It has become increasingly apparent that adaptive or induced Foxp3$^+$ Treg cells (iTreg cells) can also be generated in the periphery, differentiating from conventional T cells in a TGF-ß-dependent manner. There is some evidence in favor of a division of labor between nTreg cells and iTreg cells, with iTreg cells being important in the downmodulation of immune responses to persistent antigenic stimulation, such as the ones triggered by allergens or chronic infections.

T cell receptor (TCR) repertoire studies indicated that there is little overlap between the repertoires of Treg cells and conventional T cells. Since iTreg cells are generated from conventional T cells, their TCR repertoires are shared, at least in part. It is thus possible that the TCR repertoire overlap between Treg cells and conventional T cells reflects the presence of iTreg cells. It is also possible that factors other than the TCR determine the selection of nTreg cells. To gain some insights into the selection of nTreg cells, we generated TCR transgenic mice utilizing the TCR of Foxp3$^+$ cells. While the presence of TCRs from Treg cells resulted in an increased frequency of Foxp3$^+$ Treg cells compared to the frequency of Foxp3$^+$ cells observed in TCR transgenic mice made with conventional T cells, the number of transgene-encoded Treg cells remained exceedingly low. In fact, the vast majority of the transgene-encoded T cells were CD4$^+$Foxp3$^-$ cells, indicating that factors other than the TCR are limiting Treg differentiation, presumably the availability of the ligand recognized by Treg cells. We propose that Foxp3$^+$ regulatory T cells differentiate in small sized niches.

I5 – Autoimmunity: Clinical Aspects

Edwards J.C.W.
University College London, UK

Autoimmunity is often considered in terms of an adaptive immune response to self antigen and this is the scope that will be discussed. Adaptive responses to self might be either through T cell or B cell receptor-based recognition. In 1999 we formulated a model of autoimmune disease at UCL [1] that proposed that in most, if not all, cases disease comes about primarily through B cell receptor-based recognition, with T cell responses being either normal responses to foreign antigens or dependent on the abnormal B cell response. It also proposed that abnormal survival of autoreactive B cell clones was due to aberrant feedback signalling through both B cell receptor and T cell help. This model lead to our use of B cell depletion therapy in rheumatoid arthritis and subsequently systemic lupus erythematosus.

Clinical and pharmacodynamic studies since that time have provided support for the central hypothesis but have also raised a number of detailed issues about the complexity of B cell dynamics.

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I6 – NEW TARGETS

Soares M.P.
Instituto Gulbenkian de Ciência, Oeiras, Portugal

Abstract not available.

I7 – THERAPEUTIC TARGETING OF SIGNAL TRANSDUCTION

Bähr M.
Dept. of Neurology, University of Göttingen Medical School, Göttingen, Germany

The majority of human neurodegenerative diseases and neuroinflammatory disorders show a multifaceted pathology with signs of synaptic dysfunction, oxidative stress and axonal degeneration preceding neuronal cell loss. The latter often displays features of programmed cell death with its variants, e.g. autophagy. The pro-apoptotic signals that have been described so far seem to initiate breakdown of mitochondrial membrane potential, followed by the release of proapoptotic factors from the inner mitochondrial membrane and subsequent caspase activation.

Thus, to develop a versatile neuroprotective therapy for several different disorders one needs to focus on the maintenance of mitochondrial integrity e.g. by overexpression of antiapoptotic members of the Bcl-2 family of proteins, neurotrophic factors or other upstream regulators of mitochondrial morphology and membrane integrity. This approach, in contrast to inhibition of downstream events after AIF or cytochrome C release, apoptosome formation and Caspase activation may prove much more effective in protecting affected cells from dysfunction and subsequent cell death.

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I8 – Effector T Cells in Immune-Mediated Inflammatory Diseases

Bettelli E.
Dept. of Immunology, Benaroya Research Institute, Seattle, USA

Upon activation, CD4+ T cells differentiate in different subsets of effector T helper (Th) cells characterized by specific cytokine profile and specialized effector functions. Th cells are now divided into at least three subsets of effector T cells named Th1, Th2 and Th17 cells. Each subset of Th cells orchestrates a specific immune response, but under certain conditions, can also lead to the development of autoimmune and allergic reactions. Th17 cells have been the focus of much attention in the past few years. They are thought to contribute to host defense against extracellular bacteria and fungi, however, they are also pivotal in the development of autoimmune and allergic diseases under pathologic conditions.

In this lecture, I will review current concepts related to the differentiation of Th17 cells in mouse and humans and the unique relationship between Th17 cells and regulatory T cells. Th17 cells, like induced regulatory T cells share the requirement for TGF-b during their differentiation. Furthermore, recent evidences show that Th17 cells like regulatory T cells are more plastic than conventional Th1-Th2 cells and can acquire additional effector cytokine production. With the identification of IL-23, the Th17-IL-23 axis has emerged as a potent player in the induction of several autoimmune diseases through the promotion of tissue inflammation and the mobilization of the innate immune system. The tentative role of Th17 cells in different autoimmune and inflammatory diseases and a possible cooperation with other T cell subsets will be discussed. Together, the presentation will provide a perspective on the different effector T cells implicated in inflammatory diseases.

I9 – Immunomodulation by Sialylated IGG

Ravetch J.V.
The Rockefeller University, New York, NY, USA

The anti-inflammatory activity of intravenous immunoglobulin (IVIG) results from a minor population of the pooled immunoglobulin G molecules that contains terminal α2,6 sialic acid linkages on their Fc-linked glycans. These anti-inflammatory properties can be recapitulated with a fully recombinant preparation of appropriately sialylated IgG Fc fragments. It has now been demonstrated that these sialylated Fc's require a specific C-type lectin, SIGN-R1, (specific ICAM-3 grabbing non-integrin-related 1) be expressed on macrophages in the splenic marginal zone. Splenectomy, loss of SIGN-R1$^+$ cells in the splenic marginal zone, blockade of the carbohydrate recognition domain (CRD) of SIGN-R1, or genetic deletion of SIGN-R1 abrogated the anti-inflammatory activity of IVIG or sialylated Fc fragments.

Although SIGN-R1 has not previously been shown to bind to sialylated glycans, it has been demonstrated that SIGN-R1 preferentially binds to 2,6 sialylated Fc as compared to similarly sialylated, biantennary glycoproteins, thus suggesting that a specific binding site is created by the sialylation of IgG Fc. A human orthologue of SIGN-R1, DC-SIGN, displays a similar binding specificity to SIGN-R1 but differs in its cellular distribution, potentially accounting for the some of the species differences observed in IVIG protection. A novel antibody receptor specific for sialylated Fc has been identified as well as the initial step that is triggered by IVIG to suppress inflammation.

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I10 – Characteristics of Human Lymphoid Tissue Inducer Cell Subsets

Spits H.,[1,2] Cupedo T.,[1,3] Trifari S.,[2] Crellin N.[2]
1. Dept. of Gastroenterology, Academic Medical Center, Amsterdam, The Netherlands
2. Dept. of Immunology Genentech Inc., South San Francisco, USA
3. Erasmus Medical Center, Rotterdam, The Netherlands

Lymphoid tissue inducer (LTi) cells are required for lymph node formation during fetal development, and recent evidence implies a role in mucosal immunity in the adult. LTi cells share some phenotypic features of (immature) NK cells, however, little is known to date about the relationship between LTi cells and conventional NK (cNK) cells. Here, I discuss LTi-like populations in both human peripheral blood, bone marrow and tonsil, providing an important foundation for our understanding of LTi cell development and localization in the adult. I show that $CD127^+RORC^+$ LTi-like cells in human tonsil are precursors to $CD56^+CD127^+RORC^+NKp46^+$ cells, which together comprise a distinct and stable $RORC^+$ lineage that produces IL-22 and other cytokines that may influence the homeostasis of epithelial cells. Further, LTi-like cells and their progeny can be expanded *ex vivo* without loss of function, suggesting they constitute a stable lineage, related to but distinct from the cNK lineage. The possible role of LTi cells in the innate immune response will be discussed.

I11 – Regulation of Immune-Mediated Inflammatory Diseases – What Are the Treatment Perspectives?

Isaacs J.
Institute of Cellular Medicine, Newcastle University, Newcastle-Upon-Tyne, UK

It has become increasingly appreciated that many human autoimmune and inflammatory diseases represent defects of immune regulation rather than primarily errors of auto-recognition. Therefore, appropriate 'resetting' of immune regulation may provide a means to induce long-term disease modulation – the concept of therapeutic tolerance induction.

Whereas resetting of the immune system and therapeutic tolerance induction has been achieved in multiple animal models, the possibility has only recently been widely accepted in human disease.

Most routes to therapeutic tolerance induction target the APC-T-cell interaction. Some are antigen-specific, utilising for example autoantigen-derived peptides. This approach has been used to desensitise individuals to known allergens, for example in asthma and bee sting hypersensitivity. Equivalent approaches are now being applied in autoimmune situations, eg multiple sclerosis. In many human autoimmune diseases, however, the autoantigen is either unknown or variable. In this situation non-specific approaches have been adopted. Until recently, monoclonal antibody (mAb) targeting of T-cells has not been particularly effective in human autoimmunity. For example a large number of anti-CD4 mAbs failed to provide consistent benefit in rheumatoid arthritis. Non-activating anti-CD3 mAbs appear promising, however, and have been demonstrated to retard disease progression in a number of studies in recent onset type I diabetes mellitus. These mAbs are now starting to be applied in other autoimmune situations.

Most recently the possibility of using cellular approaches to restore immune regulation has been explored. Bespoke therapies, such as tolerogenic dendritic cells and regulatory T-cells, provide one option. However, 'off-the-shelf' options such as mesenchymal stem cells have already been used therapeutically, for example in graft versus host disease.

The single most limiting factor in the application of these therapies clinically is our lack of biomarkers of therapeutic tolerance induction. The identification and application of such markers is essential to the rational development and further clinical exploitation of immune modulating therapies.

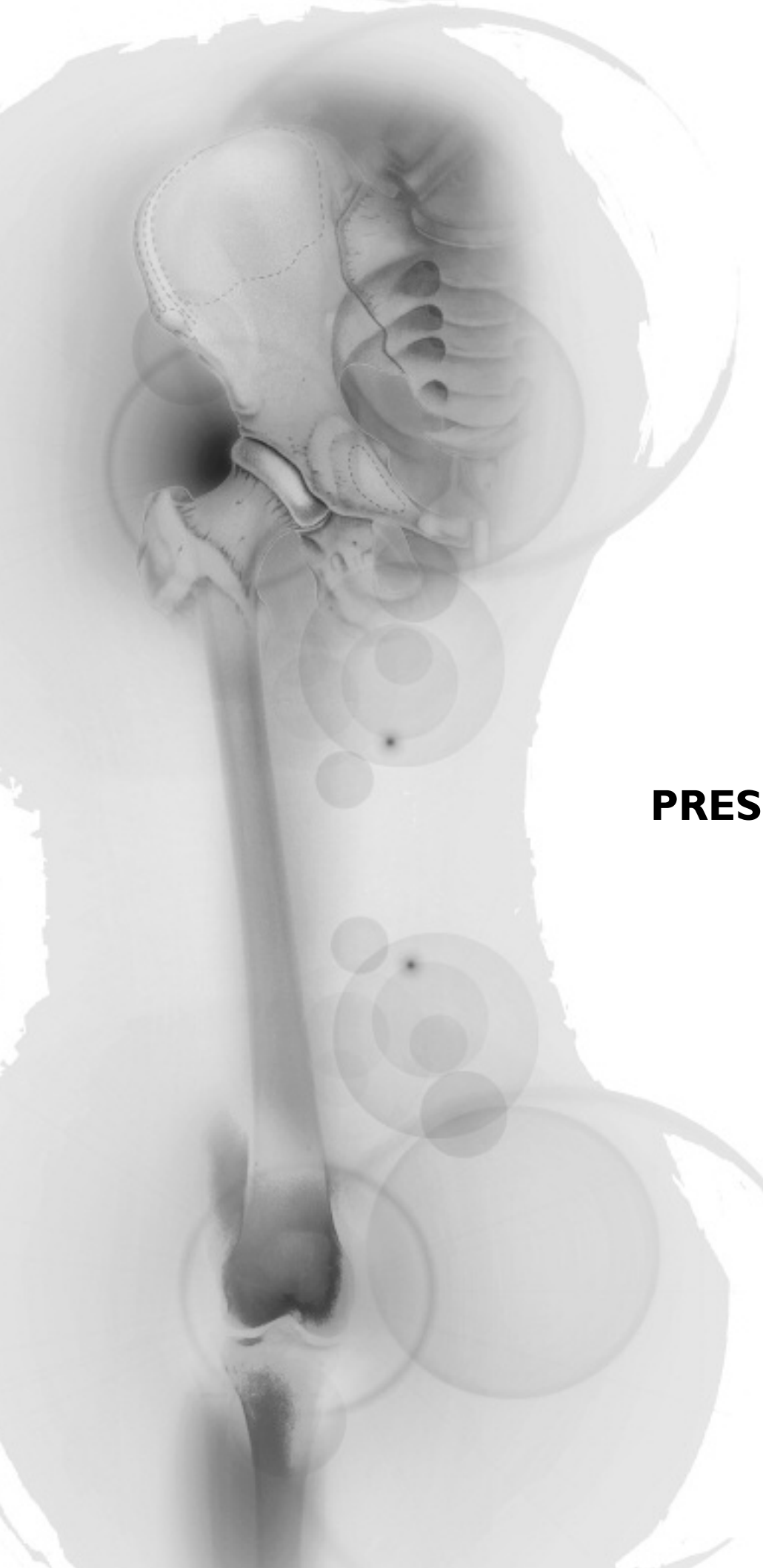
I12 – Initiation of Arthritis by Commensal Microbes

Wu J., Benoist C., Mathis D.
Dept. of Pathology, Harvard Medical School Boston, USA

K/BxN T cell receptor (TCR) transgenic mice are a model of human rheumatoid arthritis (RA). Disease is initiated by a T, then B, cell response to the ubiquitous enzyme glucose-6-phosphate isomerase (GPI). The resulting high titers of anti-GPI antibodies drive a potent effector phase, which depends on a number of innate immune system players, including mast cells, neutrophils, Fc receptors, the

complement cascade and inflammatory cytokines.

A powerful feature of this model is the ability to cleanly separate the (adaptive) initiation and (innate) effector phases, the latter being recapitulated by simply transferring serum from arthritic K/BxN mice into normal recipients. This presentation will focus on how a single species of commensal microbe, through the induction of Th17 cells in the gut, triggers arthritis in K/BxN mice.



ORAL PRESENTATIONS

ORAL PRESENTATIONS

O1 – Constitutive Expression of the Costimulatory Molecule CD70 by Non-Antigen Specific B Cells is Sufficient to Trigger T Cell-Driven Autoimmune Disease

Franco Salinas G.,[1] Cantaert T.,[1] Nolte M.,[2] Bettelli E.,[3] Kuchroo V.K.,[3] Tak P.P.,[1] Van Lier R.A.,[2] Baeten D.L.P.[1]

1. Dept. of Clinical Immunology and Rheumatology
2. Dept. of Experimental Immunology, Academic Medical Center, University of Amsterdam; Amsterdam, The Netherlands
3. Center for Neurological Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, USA

Background: B cells are critical for Experimental Autoimmune Encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG) protein. Using mice with MOG-specific B and T cells, we showed that B cells contribute to disease by T cell activation rather than by autoantibody production.

Aim: Determine whether B cells contribute to EAE by providing costimulatory signals to T cells rather than by presenting antigen. To achieve this objective, we studied the development of EAE in the presence of non-antigen specific B cells which constitutively express the costimulatory molecule CD70.

Materials and Methods: 2D2 mice (in which 95% of T cells are MOG-specific) were crossed with CD70+ mice (with transgenic expression of the costimulatory molecule CD70 under the CD19 promotor, leading to constitutive expression by B cells). Mice were scored for signs of spontaneous EAE on a 4-point scale over a 20-week period. Brains, spinal cords, optical nerves, blood and lymphoid organs were harvested for histological and functional analysis.

Results: None of the animals in the control and single transgenic groups developed signs of EAE. In contrast, 38% of 2D2+CD70+ mice developed spontaneous EAE (mean maximal clinical score 2.8; mean age of onset 73 days). Phenotypic analysis of lymphocytes revealed an increase in CD4+ T cells but a decrease in FoxP3+ regulatory T cells in the double transgenic animals. In line with our data that T cell activation by CD70 overexpression leads to massive IFN gamma production and subsequent B cell depletion, the frequency of B220+ cells and the IgG1 and IgG2b levels were decreased in 2D2+CD70+ versus 2D2+ mice, reflecting the T cell activation in the former group. Accordingly, sick 2D2+CD70+ animals displayed higher numbers of B220+ cells and antibodies levels than healthy double transgenic animals, confirming that T cell activation is the primary pathogenic role of B cells in this model.

Conclusions: The constitutive expression of the costimulatory molecule CD70 by non-antigen specific B cells is sufficient to activate pathogenic T cells and induce autoimmune disease in this model. The functional profile of these activated T cells and the corresponding immunopathology are currently under investigation.

O2 – A Gene-To-Function Analysis of the Protective IL-23R R381Q Gene Variant Reveals Impaired TH17 Response In Humans

Di Meglio P.,[1] Di Cesare A.,[1,2] Laggner U.,[1] Chu C. C.,[1] Napolitano L.,[1] Peris K.,[2] Nestle F.O.[1]

1. St. John's Institute of Dermatology, King's College London, NIHR Biomedical Research Centre and FOCIS Centre of Excellence King's College London, London, UK
2. Dept. of Dermatology, University of L'Aquila, L'Aquila, Italy

Introduction: Genome-wide association studies (GWAS) have repeatedly shown that the interleukin (IL)-23 receptor (*IL23R*) arginine-to-glutamine (R381Q) gene variant is protective against a number of immune-mediated diseases, including psoriasis and Crohn's disease.[1-3] However, the functional and immunological consequences of carrying this gene variant have not been investigated. IL-23 is highly expressed in clinical samples of psoriasis and Crohn's disease and its blockade is effective in the treatment of both diseases.[4, 5]

Moreover, IL-23 is essential for T helper 17 (Th17) cells effector function in tissue immuno-

surveillance and pathology, supporting a pivotal role for the IL-23/Th17 axis in the pathogenesis of immune mediated diseases.[6]

Aim: We investigated whether the R381Q IL-23R gene variant may protect against immune-mediated diseases by affecting IL-23/Th17 response.

Methods: We genotyped 126 healthy volunteers for the R381Q IL-23R gene variant and used 37 donors in our functional studies: 21 carrying the common G allele (group GG) and 16 heterozygous for the protective A allele (group AG). IL-23R surface and mRNA expression in CD4+ T cells were studied and number of circulating Th17 cells was determined. Th17 cells were generated from highly purified naïve T cells in the presence of TGF-β and a combination of IL-1β and IL-23 or IL-1β alone followed by IL-23 stimulation and Th17 cytokines were measured.

Results: Expression of IL-23R and frequency of circulating Th17 cells did not differ in protected AG group and common GG group. Th17 cells generated in the presence of IL-23 from group AG and GG donors produced similar amounts of Th17 cytokines. However group AG and GG donors differed in the response of committed Th17 cells to IL-23. IL-23 stimulated Th17 cells from group AG allele carriers produced less IL17A, IL-22 and IFN-γ. In addition IL-23 induced less STAT-3 phosphorylation in group AG donors compared to group GG donors.

Conclusion: Our gene to function analysis of a genetic variant associated with common immune-mediated diseases suggests a protective effect of the IL-23R R381Q in tissue-based autoimmunity, through attenuation of IL-23 induced Th17 effector function.

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O3 – The FAS -670A>G Polymorphism Influences the Susceptibility to Systemic Sclerosis Phenotypes

Broen J.,[1] Gourh P.,[2] Rueda B.,[3] Coenen M.,[2,1] Mayes M.,[2] Martin J.,[3] Arnett F.C.,[2] Radstake T.R.D.J.[1] and for the European Consortium on SSc Genetics (ECSG)

1. Dept. of Rheumatology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
2. Division of Rheumatology and Clinical Immunogenetics, Dept. of Internal Medicine, University of Texas Health Science Center at Houston (UTHSC-H), Houston, TX, USA
3. Instituto de Parasitología y Biomedicina, CSIC, Granada, Spain
4. Dept. of Human Genetics, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Authors for European Consortium on SSc Genetics (ECSG)

Hesselstrand R.,[4] Herrick A.,[5] Worthington J.,[5] Agarwal S.,[2] Tan F.,[2] Denton C.,[6] Fonseca,[6] Riemekasten G.,[7] Vonk M.C.,[1] van den Hoogen F.H.J.,[8] Kiener H.,[9] Scorza,[10] Beretta L.,[10] Simeon C.,[11] Trapiella L.,[12] Callejas-Rubio J.L.,[13] Fonollosa V.,[11] García-Hernández F.J.,[14] Aguirre-Zamorano M.A.,[15] García-Portales R.,[16] Pros A.,[17] Camps M.T.,[18] Gonzalez-Gay M.A.,[19] Airo P.[20]

4. Dept. of Rheumatology, Lund University Hospital, Lund, Sweden
5. Rheumatic Diseases Centre, University of Manchester, Salford Royal NHS Foundation Trust, UK
6. Centre for Rheumatology, Royal Free and University College Medical School, London, UK
7. Dept. of Rheumatology and Clinical Immunology, Charité University Hospital, Berlin, Germany
8. Dept. of Rheumatology, Sint Maartenskliniek, Nijmegen, The Netherlands
9. Dept. of Internal Medicine, Division of Rheumatology, University of Vienna, Austria
10. Referral Center for Systemic Autoimmune Diseases, University of Milan, Italy
11. Servicio de Medicina Interna, Hospital Valle de Hebron, Barcelona, Spain
12. Dept. of Rheumatology, Lund University Hospital, Lund, Sweden

13. Servicio de Medicina Interna, Hospital Universitario Central de Asturias, Oviedo, Spain
14. Servicio de Medicina Interna, Hospital Clinico Universitario, Granada, Spain
15. Servicio de Medicina Interna, Hospital Virgen del Rocio, Sevilla, Spain
16. Servicio Medicina Interna. Hospital Virgen de la Victoria, Málaga, Spain
17. Servicio de Reumatología, Hospital del Mar, Barcelona, Spain
18. Servicio de Medicina Interna, Hospital Carlos Haya, Malaga, Spain
19. Servicio de Reumatología, Hospital Xeral-Calde, Lugo, Spain
20. Servizio di Reumatologia ed Immunologia Clinica, Spedali Civili, Brescia, Italia
21. Dept. of Human Genetics, Radboud University Nijmegen Medical Center, The Netherlands

Objective: To investigate the possible role of the *FAS* -670A>G functional polymorphism in the genetic predisposition to systemic sclerosis (SSc) susceptibility or clinical phenotype.
Methods: We analyzed the genotype and allele frequencies of the *FAS* -670A>G polymorphism with Taqman allelic discrimination assays in six case-control sets of European ancestry and three cohorts resident in the USA, composed of 2900 SSc patients and 3186 healthy controls.
Results: In the British, Italian and American white cohort we observed an association of the *FAS* -670G allele with lcSSc (respectively p=0.049; OR 1.25; 95%CI; 1.00-1.60 and p=0.045; OR 1.43; 95%CI; 1.00-207 and p=0.036; OR 1.18; 95%CI 1.01-1.39). A meta-analyses comprising all 9 cohorts revealed an association with the FAS -670G allele and *FAS* -670GG genotype (both; p=0.036 allele; OR 1.10 95%CI 1.01 to 1.21 and genotype OR 1.13 95%CI 1.01 to 1.27) and the lcSSc phenotype. In a meta-analyses considering only Caucasian individuals we found that both the *FAS* -670G allele as well as the *FAS* -670GG genotype were associated with lcSSC (genotype; p= 0.017; OR; 1.16 95%CI 1.03 to 1.31 and allele p= 0.020;OR 1.12; 95%CI 1.02-1.24). In addition, a recessive model of the -670GG genotype revealed a strong association with SSc, lcSSc and ACA+ lcSSc (respectively, p=0.004; OR 1.23; 95%CI 1.07 to 1.41, p=0.0003; OR 1.33; 95%CI 1.14 to 1.56) and p=0.0002; OR 1.45; 95%CI 1.19 to 1.76).
Conclusion: Our data show that the FAS -670A>G polymorphism plays a role in lcSSc susceptibility, a trend observed in other auto-immune diseases as well.

O4 – Mast Cells Contribute to Synovial Inflammation in Non-Psoriatic And Psoriatic Spondyloarthritis

Noordenbos T., Yeremenko N., Cantaert T., Teitsma C., van de Sande M., Tak P.P., Canete J., Baeten D.L.P.
Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam, The Netherlands; Rheumatology, Hospital Clinic de Barcelona and IDIBAPS, Barcelona, Spain

Objective: We recently observed a striking synovial infiltration with cells positive for C-kit, a marker for mast cells and hematopoietic stem cells, in psoriatic arthritis (PsA). As mast cells have potent inflammatory functions, including the production of TNF, we performed a systematic analysis of C-kit positive cells in different forms of chronic inflammatory arthritis.
Materials and Methods: Synovial tissue biopsies from active rheumatoid arthritis (RA) (n=21), non-psoriatic spondyloarthritis (SpA) (n=16), and PsA (n=23) was stained by immunohistochemistry and double immunofluorescence. Synovial fluid (SF) from RA (n=18), SpA (n=19), and PsA (n=16) was analyzed by ImmunoCap and ELISA. The effect of C-kit inhibition by imatinib mesylate on proinflammatory cytokine production was tested in vitro on fresh SpA synovial biopsies.
Results: C-kit positive mononuclear cells were found in the synovial sublining in all disease groups but were significantly increased in SpA (p=0.010) and PsA (p=0.001) versus RA despite similar levels of global inflammation as reflected by CD3, CD20, and CD68 staining. Double stainings confirmed that C-kit positive cells were not hematopoietic stem cells but mast cells. SF levels of SCF, IL-3, and IL-33, all factors involved in chemotaxis and differentiation of mast cells, as well as sST2, the soluble decoy receptor for IL-33, were similar in all groups. Most C-kit positive mast cells in SpA synovium were degranulated as indicated by double staining with toluidine blue and anti-tryptase and by SF analysis for mast cell products. Interestingly, the synovial infiltration with C-kit positive cells in SpA persisted despite successful treatment with TNF blockers. However, C-kit inhibition in vitro strongly reduced the production (mRNA by qPCR) and secretion (protein by ELISA) of IL-6 and IL-8 by synovial biopsies, suggesting that mast cells contribute to the ongoing inflammatory process.
Conclusion: There is an increased synovial infiltra-

tion with and degranulation of C-kit positive mast cells in non-psoriatic and psoriatic SpA. Inhibition of C-kit in vitro leads to a reduction of proinflammatory cytokine production by synovial biopsies. These data suggest a role for mast cells in driving and/or sustaining the synovial inflammation in SpA.

O5 – Differential Expression Of CD5, CD21 And CD1D in Peripheral B Cells of Healthy Controls and Spondyloarthritis Patients

Cantaert T.,[1] Tiersma Y.,[1] Ambarus C.A.,[1] Paramarta J.E.,[1] De Rycke L.E.,[1] Tak P.P.,[1] Baeten D.L.P.[1]

1. Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, The Netherlands

Introduction: The role of B cells in immune mediated rheumatic diseases is still unknown. In rheumatoid arthritis (RA), a subset of patients produces autoantibodies such as anti-citrullinated protein antibodies (ACPA). In Spondyloarthritis (SpA) patients no autoantibodies are detectable and these patients do not appear to respond to B cell depletion.

Aim: By investigating different B cell subsets in RA and SpA, we will gain more insight in the role of certain B cell subsets in the pathophysiology of RA and SpA.

Patients and Methods: We collected PBMC of 11 SpA patients, 11 RA patients (6 ACPA+ and 5 ACPA) and 13 healthy controls. None of the patients received biological therapy.

Subsets were analyzed using eight color flow cytometry (FacsCanto, BD) and FlowJo software. Statistical analysis was performed using Kruskal-Wallis test for multiple comparisons of non-parametric data.

Results: Total numbers of B cells were similar between the 3 groups. The number of naïve (IgD+/CD27-), memory (IgD-/CD27+), and marginal zone like (IgD+/CD27+) B cells were not different. Dividing the B cells according to CD38/IgD expression (Bm subsets), no difference could be observed. CD38 expression (activated B cells) was higher in ACPA- RA patients versus ACPA+ RA patients (P = 0.009). CD21 expression is lowered in memory B cells in SpA compared to HC (P =0.017) and in ACPA+ patients compared to ACPA- patients (P = 0.03). Furthermore, CD1d expression is lower in SpA B cells compared to HC (P = 0.003).

Interestingly, CD5 expression (B1a subset) is elevated in SpA B cells versus HC B cells, most pronounced in the IgD+/CD27+ subset (P = 0.012). These B cells mostly reside in the peritoneum and are responsible for the production of natural antibodies.

Conclusion: No differences were observed between naïve, memory and IgD+/CD27+ B cell subsets. Interestingly, SpA patients show differential expression of specific markers such as CD5, CD1d or CD21.

O6 – TNF Blockade Impairs T Cell Dependent Antibody Responses

Franco Salinas G.,*[1] De Rycke L.,*[1] Cantaert T.,[1] Remans P.,[1] van der Burg M.,[2] Barendregt B.,[2] Tak P.P.,[1] Baeten D.L.P.[1]

1. Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Amsterdam

2. Dept. of Immunology, Erasmus Medical Center, Rotterdam, The Netherlands

*Both authors contributed equally to this work

Objective: TNF blockade in spondyloarthritis (SpA) induces antibodies specific for double stranded DNA, which is a T cell independent (TI) antigen. As these antibodies were restricted to the IgM isotype and no antibodies to T cell dependent (TD) antigens were induced, we investigated here if TNF blockade impairs the induction and maturation of TD humoral responses.

Methods: 30 SpA patients (20 treated with TNF blockade, 10 untreated controls) were vaccinated with a TD vaccine to Hepatitis B and a TI vaccine to *S. pneumoniae*. Another 10 SpA patients treated with infliximab were vaccinated with a TD vaccine to *S. preumoniae*.

Serum and PBMCs were collected before and after vaccination. Vaccine-specific antibody titters were measured by ELISA. B and T cell populations were evaluated by flow cytometry.

Somatic hypermutation was determined by the Igκ REHMA assay (Anderson, Blood 2005).

Results: IgM and IgG responses against TI antigens were moderately decreased in anti-TNF treated patients compared to controls but were still robust. In contrast, IgG responses against TD antigens were almost completely absent in treated patients. The greater suppression of TD versus TI responses

by TNF blockade was confirmed by lower IgG titers with TD versus TI vaccins against anti-*S.pneumoniae*. Phenotypic analysis showed a normal number of B cells but a decrease in naïve and memory CD4+ T cells upon TNF blockade. Within the B cell population, TNF blockade significantly increased the frequency of memory B, which displayed an activated phenotype with increased expression of CD40 and HLA-DR. In parallel, however, TNF blockade decreased the frequency of CD138+ plasmablasts, suggesting a defective maturation towards antibody-producing cells. Moreover, TNF blockade significantly decreased the degree of somatic hypermutation as evidenced by Igκ REHMA analysis of peripheral blood B cells before and after treatment.

Conclusion: TNF blockade severely impairs TD humoral responses by interfering with the affinity maturation and differentiation of activated B cells towards antibody producing cells.

O7 – Complete T- and B-Cell Receptor Repertoire Analysis in Rheumatoid Arthritis Using Massive Parallel Sequencing

Klarenbeek P.L.,[1] Doorenspleet M.E.,[1] van Schaik B.D.C.,[2] Herenius M.M.,[1] Jakobs M.E.,[3] Cantaert T.,[1] Baeten D.L.P.,[1] van Kampen A.H.C.,[2] Baas F.,[3] Tak P.P.[1], de Vries N.[1]

1. Dept. of Clinical Immunology and Rheumatology
2. Dept. of Clinical Epidemiology, Biostatistics and Bioinformatics,
3. Neurogenetics Lab., all at AMC/University of Amsterdam, Amsterdam, The Netherlands

Background: T-cells and B-cells are likely to play important roles in the pathogenesis of rheumatoid arthritis (RA). Previous attempts to investigate the roles of T- and B-cell clones in RA by screening the T-/B-cell receptor (TCR/BCR) repertoires were hampered by the sheer size and complexity of the repertoires. Current techniques are unable to analyse whole repertoires in sufficient detail, are vulnerable to artefacts, and do not provide quantitative data. Here, we used our newly developed protocol based on massive parallel sequencing (MPS) which overcomes current limitations and produces the DNA-sequence of >100.000 receptors in a single experiment. Using this technique we performed the first quantitative, high-resolution analysis of the complete TCR and BCR repertoires in an RA patient.

Objectives: Describe the complete BCR and TCR repertoires in synovial tissue (ST) and peripheral blood (BL) samples of an RA-patient and screen for dominant T- and B-cell clones.

Methods: mRNA was isolated from BL and ST, simultaneously taken from an aCCP+ RA-patient with active disease despite treatment with methotrexate. A multiplex linear amplification with primers for all V(ariable)-families of the receptor beta-chain (TCR) or heavy-chain (BCR) was performed. The samples were analysed on a Genome Sequencer FLX (Roche) resulting in 14000 reads/samples for TCR and 35000 for BCR analysis, each containing the full CDR3 sequence to identify clones. Bioinformatics algorithms were used to identify gene segments and correct for sequencing errors.

Results: TCR-repertoire: in ST most TCRs contained a Vbeta6(46%), 10(19%), and 27(13%) gene segment, while in BL V29(32%) and 7(26%) were most frequent. The TCR repertoire was dominated by low-frequency clones (>95%), both in the BL and ST. Several clones were clearly expanded (up to 217 and 121 copies/clone for BL and ST). However, the dominant clones in ST were different from those in BL, as compared by V-segment and CDR3 sequence.

BCR-repertoire: the ST sample showed preferential usage of the small V2,5,6,7 families (total 50%) when compared to published data in BL[1](20%). Several clearly expanded clones (up to 1892 copies) were found against a background of low-frequency clones.

Conclusions: This is the first high-resolution analysis of the TCR and BCR repertoire in RA, providing detailed insight in the presence of T- and B--cell clones. We found clear differences between the TCR-repertoire in ST compared to BL in an RA-patient. Several expanded clones were found only in ST suggesting proliferation or local retention of T-cells. The BCR-repertoire also showed expanded clones within the ST. Further studies will elucidate the role of these clones in RA.

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O8 – Development Of Biliary Fibrosis In FRA-1 Transgenic Mice

Kireva T.,[1] Erhardt A.,[2] Tiegs G.,[2] Aigner E.,[3] Datz C.,[3] Tilg H.,[4] Moschen A.,[4] Voll R.E.,[1] Distler J.H.,[1] Schett G.,[1] Zwerina J.[1]

1. Dept. of Internal Medicine 3, University of Erlangen-Nuremberg

2. Dept. of Experimental Immunology, University of Hamburg, Germany
3. Dept. of Internal Medicine, Hospital Oberndorf
4. Christian Doppler Research Laboratory for Gut Inflammation, Innsbruck Medical University, Innsbruck, Austria

Introduction: Chronic diseases of the biliary system can cause fibrosis and eventually progression to liver cirrhosis. Chronic biliary disorders are a heterogeneous group including autoimmune diseases such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) as well as ischemic, toxic, infectious and metabolic disorders. Despite the heterogeneous origins, cholangiopathies share pathogenetic mechanisms. Inflammatory infiltrates, bile duct proliferation and destruction and fibrosis are common features.
Aim: To define a new mouse model of a cholangiopathy leading to liver fibrosis in *Fra*-1 tg mice.
Methods: *Fra*-1 tg mice and their wildtype littermates were sacrificed at different ages. Alterations of liver morphology and liver and blood cell composition were investigated by histological stainings and by flow cytometry, respectively. Hepatic collagen content was determined biochemically and morphometrically. Transcript levels of fibrosis-related genes and MMP activities were quantified and immunohistochemical analysis additionally applied.

Furthermore we investigated the role of lymphocytes in this model by crossing *Fra*-1 tg mice with *Rag2*$^{-/-}$ mice.
Results: *Fra*-1 tg mice spontaneously develop biliary fibrosis preceded by ductular proliferation and infiltration of inflammatory cells. As determined by immunohistochemistry, Fra-1 protein is present in cholangiocytes and inflammatory cells within the liver. The inflammatory infiltrate is composed of neutrophiles, granulocytes, lymphocytes and macrophages. Hepatic FACS analysis revealed a strong increase in activated T cells and decreased B cells, NK and NKT cells in *Fra*-1 tg mice as compared to wildtype mice.

Moreover *Fra*-1 tg mice develop severe biliary fibrosis with a time-dependent increase in hepatic collagen content and increase in relative mRNA expression of profibrogenic genes.

Attenuation but not complete prevention of collagen accumulation in liver was observed in the *Fra*-1 tg x *Rag2*$^{-/-}$ mice. However, transplantation of *fra*-1 tg bone marrow cells into wildtype mice could not induce disease.
Discussion: *Fra*-1 tg mice spontaneously develop a progressive biliary disease. These mice are an attractive model for the investigation of cholangiopathies and their interaction with the immune system. Furthermore they are a suitable model for testing potential antifibrotic treatments of biliary and liver fibrosis.

O9 – The Role of P38MAPKα in Rapid Progressive Crescentic Glomerulonephritis

Mueller R.,[1] Amann K.,[2] Heeringa P.,[3] Hugo C.,[4] Daniel C.,[4] Baum W.,[1] Schett G.,[1] Zwerina J.[1]
1. Dept. of Internal Medicine 3, Rheumatology and Immunology
2. Dept. of Nephropathology, University of Erlangen-Nuremberg, Erlangen, Germany
3. Dept. of Pathology, University Medical Center Groningen, Groningen, The Netherlands
4. Dept. of Nephrology and Hypertension, University of Erlangen-Nuremberg; Erlangen, Germany

Introduction: The p38 MAPK signaling cascade is a signalling pathway involved in acute and chronic inflammatory diseases such as crescentic glomerulonephritis. It is however unclear which of the four isoforms of p38 (α,β,γ and δ) is the most important one.
Aim: To identify the major isoform of p38MAPK involved in disease progression in rapid progressive crescentic glomerulonephritis (crGN).
Methods: An experimental model of crescentic glomerulonephritis (anti-glomerular basement membrane GN) was induced in p38α conditional knockout mice (p38$^{f/f}$ x Mx1-Cre transgenic mice) and wildtype mice. Using RNA and protein methods including immunoprecipitation, expression pattern and activation of the four p38MAPK isoforms were analyzed. We also analyzed upstream and downstream kinase activation of p38MAPK. All biochemical analyses like WB, IP, pPCR were also performed in a murine podocyte cell line to identify the role of podocytes in p38 MAPK signaling. The course of experimental crGN was investigated using morphological assessment of kidneys and immunostaining to detect the influx of neutrophil granulocytes, T-cells, B-cells and macrophages in glomeruli and inflamed kidney tissue. Blood urea nitrogen (BUN) was evaluated to determine the kidney function during the disease.
Results: After induction of crGN in wildtype mice,

p38MAPK was early and sustained activated in the anti-GBM model of glomerulonephritis. Wildtype mice developed end-stage renal failure associated with glomerular crescents. In comparison to wildtype mice, *p38$^{f/f}$Mx1cre*tg mice had an improved survival rate and kidney function (BUN level) during investigation period. Stimulation of podocytes in vitro with inflammatory stimuli such as TNF led to a fast activation of p38 MAPKα but not the other isoforms.

Conclusion: Our results demonstrate that p38α is the most important isoform of p38 signaling in crGN.

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O10 – Anti-Tnf Prolongs Cardiac Allograft Survival by Blocking the Induction of Alloantibodies

Brouard S.,[1] Franco Salinas G.,[2] Jovanonic V.,[1] Moizant F.,[1] Tak P.P.,[2] Soulillou J.P.,[1] Baeten D.L.P.[1,2]

1. INSERM U643, Nantes, France

2. Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam, The Netherlands

Objectives: Based on our observations of IgM but not IgG anti-dsDNA antibody induction in patients treated with TNF blockers, we proposed the hypothesis that TNF blockade interferes with the induction of T cell dependent humoral responses. This study aimed to assess this hypothesis by assessing the induction of alloantibodies in a rat cardiac allograft model.

Materials and Methods: LEW.1W hearts were ectopically transplanted in LEW.1A rats, which were treated with anti-rat TNF or control antibody (3G8). Graft rejection was monitored clinically and serum was obtained every 5 days until day 25. Transplanted hearts were obtained 5 days after transplantation and were assessed by histology, immunohistochemistry, and quantitative RT-PCR for cytokines (Th1/Th2), TLRs, and regulatory molecules.

Results: Graft survival in the LEW.1W to LEW.1A rat allotransplantation model was prolonged from 6 days to 13 days by a single IP injection with anti-TNF (8 mg/kg) at day 0.

Upon multiple injections (day 0, 3, and 6), the mean graft survival was further prolonged to 23 days. Higher dosage of anti-TNF (15 mg/kg) or concomitant treatment with suboptimal doses of cyclosporin or rapamycin did not have a significant additional effect.

At day 5, histology of the graft showed a better conserved histological architecture in anti-TNF treated recipients compared to controls (Banff grade 2-3A versus 3A-3B). Accordingly, immunohistochemical analysis showed a lower number of infiltrating leucocytes in the anti-TNFalpha treated grafts (p=0.015), with a similar trend for T lymphocytes, B lymphocytes, and macrophages. Of major interest, IgG deposition was significantly lower in anti-TNFalpha treated grafts than control grafts (p=0.001), without significant differences for IgM deposition.

ELISA analysis of serum showed a clear induction of alloantibodies in the control-treated rats from day 5 on. This induction was significantly impaired by single anti-TNF treatment and completely blocked by triple anti-TNF injection.

In contrast with the alloantibody data, TNF blockade did not affect the Th1/Th2 balance, TLRs, or the expression of the regulatory molecules TGF-beta, IDO, HO-1, and FoxP3.

Conclusions: TNF blockade completely blocks the induction of alloantibodies, which results in a significant prolongation of graft survival in this allotransplantation model. TNF blockade may have a similar effect on humoral responses in other situations, including clinical treatment of IMID patients.

O11 – Characterization and Differentiation Of Human Il-22-Producing Cd4+ T Cells

Coffre M., Placek K., Maiella S., Bianchi E., Rogge L.

Institut Pasteur, Immunoregulation Unit and CNRS URA 1961, Dept. of Immunology, Paris, France

In addition to IL-17A and IL-17F, mouse Th17 cells also produce the IL-10-related cytokine IL-22. Th17

cell-derived IL-22 has been shown to mediate IL-23-induced skin inflammation in a model of psoriasis, indicating a proinflammatory function of IL-22. However, similar to IL-10, IL-22 has also potent anti-inflammatory activities in models of acute hepatitis and inflammatory bowel disease. These studies showed that, although IL-17 and IL-22 are coexpressed by inflammatory Th17 cells in the mouse, these cytokines have diverging activities on inflammatory responses. Recent reports have suggested that IL-22 may not be a Th17 "signature" cytokine, but may define a separate T helper cell subset.

We have determined which human $CD4^+$ T cell subset(s) secrete IL-22 and how IL-22 expression is regulated during $CD4^+$ T cell differentiation. Analysis of cytokine production by distinct populations of naïve and memory $CD4^+$ T cell populations revealed that similar to IL-17, most of the IL-22-secreting $CD4^+$ T cells were memory cells expressing the chemokine receptor CCR6. Consistent with the notion of IL-22 being a Th17 cytokine, analysis of cytokine production at the single cell level revealed that approximately half of IL-17--producing cells also secreted IL-22. IL-22 was also produced by a fraction of Th1 cells. Of note, a small proportion of $CD4^+$ T cells produced IL-22, IL-17 and IFN-γ. Analysis of IL-22 expression in highly purified Th1 and Th17 cells, isolated by cell sorting based on the secretion of IFN-γ and IL-17, respectively, provided further evidence that IL-22 expression is not restricted to Th1 or Th17 cells. The majority of IL-22-producing cells, however, produced neither IL-17 nor IFN-γ. IL-22-producing cells also secreted TNF-α, indicating a pro-inflammatory role of IL-22-producing $CD4^+$ T cells from peripheral blood.

T cell receptor signaling was sufficient to induce IL-22 expression in cord blood-derived naïve $CD4^+$ T cells. Addition of Th1-inducing or various pro-inflammatory cytokines increased IL-22 expression, while TGF- β and cyclosporine A strongly inhibited IL-22 production. In conclusion, our results indicate that IL-22-producing cells represent a heterogeneous cell population that partially overlaps with Th1 and Th17 subsets.

O12 – TREGS: T CELL REGULATORS OF BONE

Zaiss M.M.,[1] Sarter K.,[1] Frey B.,[2] Hess A.,[3] Zwerina J.,[1] Nimmerjahn F.,[1] Engelke K.,[4] Kollias G.,[5] Douni E.,[5] Voll R.,[1] Burkhart M.,[6] Rauh M.,[7] Kroczek R.A.,[8] Ziegler S.,[9] Schett G.,[1*] David J.P.[1]

1. Dept. of Internal Medicine 3, Rheumatology and Immunology
3. Institute of Experimental and Clinical Pharmacology and Toxicology
4. Institute of Medical Physics, University of Erlangen-Nuremberg; Erlangen, Germany
5. Institute of Immunology, Alexander Fleming Biomedical Sciences Research Center, Vari, Greece
6. Scanco Medical Inc., Brüttisellen, Switzerland
7. Dept. of Paediatrics, University of Erlangen-Nuremberg, Erlangen, Germany
8. Molecular Immunology, Robert-Koch Institute, Berlin, Germany
9. Benaroya Research Institute at Virginia Mason, Seattle, USA

Introduction: Regulatory T cells (Tregs) can block the differentiation of the bone resorbing osteoclasts *in vitro* (1). This inhibition required cell to cell contact and was mediated via CTLA-4 (2). Thus, while T cells activation is known to trigger bone loss, our *in vitro* data suggested that immune regulation could actively protect bone.
Aim: To unravel the *in vivo* role of Tregs in controlling bone homeostasis in physiological and pathological conditions.
Methods: Bone parameters of mice with increased numbers of Tregs (*foxp3*tg mice) or *RAG1*$^{-/-}$ mice after adoptive Tregs transfer model were analyzed. Histological parameters such as osteoclast numbers, osteoclast surface per bone surface and osteoblast numbers as well as mineral apposition rate (MAR) and immunochemical lymphocytes stains have been performed. Ovariectomy was used as model for post-menopausal osteoporosis. Bone marrow chimeras with *hTNF*tg mice as recipients and f*oxp3*tg and *scurfy* mutant as bone marrow donors were analyzed to address the function of Tregs in arthritis-mediated bone destruction. Bones of mice deficient for co-stimulatory T cell receptor molecules on Tregs or on osteoclast precursors (*CD28*$^{-/-}$, *CD80/86*$^{-/-}$, *ICOSL*$^{-/-}$ and *ICOS*$^{-/-}$) were studied in regard to there suppressive effect of Tregs on osteoclast differentiation *in vitro* and in *vivo*.
Results: *foxp3*tg mice developed high bone mass and were protected from osteoporosis, inflammatory osteopenia and arthritic bone destruction, whereas *foxp3*-deficiency enhanced local and systemic bone loss in *hTNF*tg bone marrow chime-

ra mice. In addition, an increased systemic bone mass was observed following adoptive Tregs transfer into *RAG1*$^{-/-}$ mice.

Protective effects of Tregs on bone were associated with decreased osteoclast numbers *in vivo*. *In vitro*, binding of Tregs to CD80/CD86 on osteoclast precursors was essential for inhibiting osteoclast differentiation. Importantly, *CD80/86*$^{-/-}$ mice developed osteopenia due to increased osteoclast differentiation, whereas other costimulation mutants did not show any bone phenotype. An increased rate of apoptosis in osteoclast precursors through IDO upregulation and increased tryptophan catabolism mediated the suppressive effects of Tregs, after CTLA-4 - CD80/86 binding, on osteoclast differentiation.

Conclusion: These results demonstrate that Tregs control bone resorption through direct inhibition of osteoclast differentiation and therefore preserve bone mass during physiological and pathological bone remodeling.

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O13 – Decreased Numbers of Circulating Dendritic Cells And Defective Suppressive Function Of T Regulatory Cells in ANCA-Associated Vasculitis

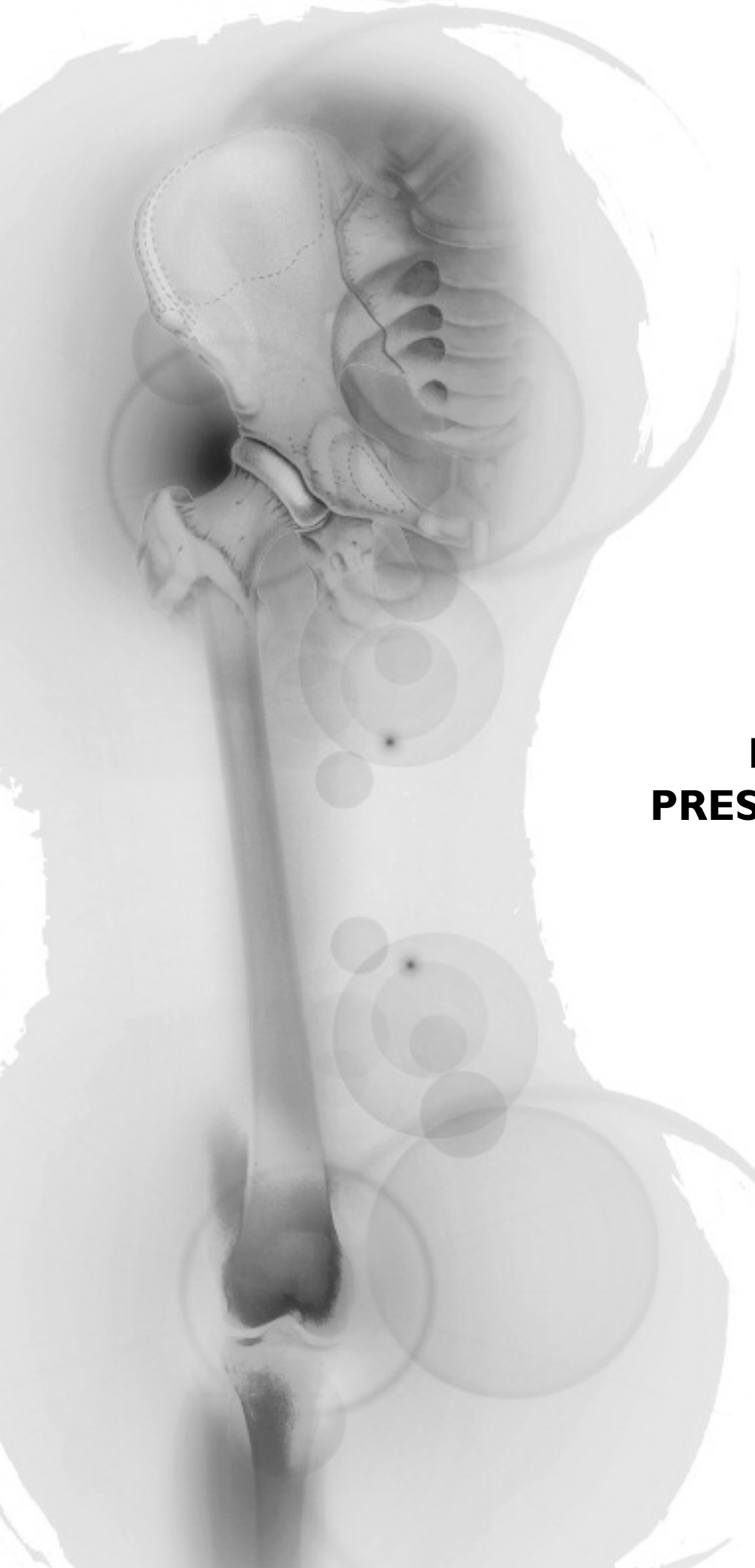
Rimbert M.,[1,2,3] Hamidou M.,[4,5] Puechal X.,[6] Audrain M.,[1] Braudeau C.,[1] Teixeira L.,[7] Josien R.[1,2,3,4]
1. CHU Nantes, Laboratoire d'Immunologie
2. ITERT-Uro-Néphro (IUN), Nantes
3. INSERM U643, Nantes
4. Université de Nantes, Faculté de Médecine, Nantes
5. CHU Nantes, Service de Médecine Interne, Nantes
6. CH Le Mans, Service de Rhumatologie, Le Mans
7. AP-HP, Hôpital Cochin, Service de Médecine Interne, Paris, France

Introduction: Anti-neutrophil-Cytoplasmic Antibodies (ANCA) associated vasculitis (AAV) are small vessel vasculitis including Wegener's granulomatosis, microscopic polyangiitis and Churg-Strauss syndrome. Even if the role of the ANCA in the inflammatory process is well known, AAV pathogenesis is still unclear. Dendritic cells (DC) play a pivotal role in controlling the immune response in both physiological and pathological conditions. DC also control T regulatory cells (Treg) function which are critically involved in autoimmune diseases. The aim of our study was to assess the frequency and phenotype of peripheral blood DC subsets and the frequency and suppressive function of $CD4^+$ $CD25^+$ $CD127^{low/-}$ T cells (Treg) in patients with AAV either during the acute phase or remission.

Methods: Blood samples from 19 untreated patients in acute phase (BVAS>6), 17 patients in remission (with low dose steroids <10mg and without immunosuppressive treatment) rand 18 age-matched healthy controls were analysed. DC and Treg were measured by flow cytometry and DC were characterised for maturation and homing potential with CD86, CCR7 and CD62L. Suppressive function of FACS-sorted Treg was determined by coculture assay.

Results: We observed significant decrease in total DC numbers in acute phase versus healthy control and remission (8 DC/µL vs 20 DC/µL, p<0,0001; 8 DC/µL vs 15 cells/µL p=0.0006). DC numbers were also reduced in patients in remission as compared healthy control (15 cells/µL vs 20 DCs/µL; p= 0.0391). This diminution was observed at the same extent in pDC and mDC. Levels of CD86 and CCR7 were not significantly different between the 3 groups. However, a significant increase in CD62L expression levels was observed on DC from acute phase patients as compared to remissions and controls. Treg numbers were slightly but significantly reduced in AAV patients as compared to healthy controls. In contrast, Treg from acute phase patients exhibited a dramatic decrease of suppressive activity as compared to controls group (8% vs 78%. p<0.001) and remission group (8% vs 36%. p=0.0364). Treg suppressive function from the remission group was also reduced as compared to control group (36% vs 78%. p=0.0004).

Conclusion: We conclude that DC numbers as well as Treg suppressive activity are significantly reduced during AAV in a disease activity-specific manner. The decrease in DC numbers may reflect their recruitment in secondary lymphoid organs as suggested but the increase in CD62L expression on DC. The decrease in Treg suppressive function might be related to changes in DC function and might be an important defective tolerance checkpoint in AAV.



POSTER PRESENTATIONS

POSTER PRESENTATIONS

P1 – Compromised function and increased frequency of IL- 5 and T-regulatory cells in patient with Churg-Strauss syndrome with different degrees of eosinophilia

Lishchuk-Yakymovych K., Chopyak V., Potomkina H., Synenkyy O., Pukalyak R., Valchuk I.
Lviv National Medical University named after Danylo Halytsky, Dept. of Clinical Immunology and Allergology, Lviv; Lviv Oblast Clinical Hospital, Rheumatology Dept., Lviv, Ukraine

Introduction: Churg-Strauss syndrome is a rare disorder characterized by hypereosinophilia and systemic vasculitis occurring in patients with asthma and allergic rhinitis Regulatory T cells (Tregs) are essential in the control of tolerance. Evidence implicates Tregs in human autoimmune conditions.
Aim: Here we investigated their role in Churg-Strauss syndrome with different stage of eosinophilia and IL-5 level.
Patients and Methods: Under our supervision there were 30 patients (16 women and 14 men) in age from 19 to 78 years. In all patients preliminary was diagnosed systemic vasculitis and then was verified Churg-Strauss syndrome accordant to the criteria of American College of Rheumatology (1990). Patients were subdivided as having CSS with light eosinophila (absolute eosinophil count > or = 500 cells/microL) (n = 12) or CSS with severe eosinophilia (absolute eosinophil count > or = 1500 cells/microL) (n = 18). Further subdivision was made between early CSS (5,0±2,3 years) (n = 14) and late CSS (15,0±3,5 years) (n = 16) based upon the duration of disease. 31 controls were studied for comparison.

CD3+ cells were isolated using FACS and subsequently studied for the expression of CD4, CD8, CD25, CD71, CD HLA-DR using flow cytometry. IL-5 as selective cytokine for eosinophils production was performed using ELISA.
Results: Increased level of IL-5 with severe eosinophilia in patients with CSS was correlated with higher CD71 and CD HLA-DR surface expression with a significant ($P < 0.05$). And cell surface antigen CD25 was correlated with some increasing of IL-5 level with light eosinophilia in patients with Churg-Strauss syndrome with a significant ($P < 0.01$). The frequency of CD4+CD25+ was highly increased in patients with late CSS. Although the expression of CD25 and IL-5 level was comparable between groups.
Conclusions/Significance: These results indicate that IL-5 could be dominant in the CSS development and its future complication. These data suggest that a defective T-regulatory function may underlie the immune dysfunction in Churg-Strauss syndrome.

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P2 – The Involvement of the Complement System in the Development of Autoinflammatory Diseases

Mkrtchyan G.M., Hovhannisyan L.P., Khazan N., Boyajyan A.S.
Institute of Molecular Biology of Armenian National Academy of Sciences, Yerevan, Armenia

Introduction: Familial Mediterranean fever (FMF) is the most prevalent member of autoinflammatory diseases worldwide, occurring mainly in Mediterranean populations. FMF is defined as illnesses caused by primary dysfunction of the innate immune system. It is characterized by unexplained recurrent attacks of inflammation, which respond favorably to colchicine treatment. Although the possibility of multiple immunological mechanisms has been studied, however the actual molecular mechanism involved in the development of the inflammatory response occurring in FMF is unresolved.
Aim: The present study evaluates the role of complement system in the pathogenesis of FMF and the influence of colchicine therapy on these reactions.
Patients and Methods: As the indicators of the inflammatory response, the hemolytic activities of alternative (AH50) and classical (CH50) pathways of the complement and the activities of the key complement components C3 were determined in the serum of 52 FMF patients with and without colchicine treatment. The control group consisted of 26 sex- and age-matched healthy volunteers without FMF positive family history. A hemolytic assay was based on the standard 50% complement hemolysis test.
Results: In comparison to healthy subjects, significant increase of the CH50 and the C3 were detected in the serum of colchicine-free patients. But in the serum of patients who were receiving regular colchicine treatment the differences of these parameters were not significant. In the serum of colchicine-free FMF patients the differences of these parameters were significantly higher, than in patients, who received regular colchicine treatment.

However, a decrease of the hemolytic activities of complement by alternative pathway was found in both cases.
Conclusion: Finally, we assessed the effect of colchicine treatment on the decrease of the serum hemolytic activity of complement classical pathway. By contrast, we observed a significant difference between treated and untreated patients, suggesting that the therapeutic effect of colchicine occurs by inhibiting the activation of complement system by classical pathway. Altogether, our results suggest that regular colchicine treatment results in suppression of hyperactivation of the complement system in patients with FMF and brings it to a normal level. In addition, these results raises the possibility that colchicine might be potential immunomodulating drug.

P3 – A Whole Blood Assay to Assess the Ex Vivo Responsiveness Of Blood PDC, BDCA1+ and BDCA3+ Dendritic Cell Subsets To TLR Ligands

Braudeau C., Josien R., Neel A., Rimbert M.
CHU Nantes, INSERM U643 and Laboratory of Immunology, Nantes, France

Blood dendritic cells (DC) encompass plasmacytoid DC (pDC) and several subsets of so-called conventional DC (cDC): BDCA1$^+$ DC (CD1c), BDCA3$^+$DC (CD141, thrombomodulin) and CD16$^+$ DC which also express CD14. Here we set up an whole blood assay to assess DC responsiveness to various TLR ligands. Heparanized blood was incubated with various TLR ligands in the presence of brefeldin A and then stained with CD45, Lin cocktail, HLA-DR, CD11c and CD123 mAb followed by intracellular cytokine staining (IL-12p40, TNF-α and IFNα). cDC were separated with BDCA1 and BDCA3 mAb whereas CD16$^+$ DC were excluded from this analysis. We demonstrate that, as expected, cDC (CD11c$^+$CD123$^-$) responded to to TLR 1, 2, 3, 4, 5, 6 and 8 but not 7 and 9 and produce IL12-p40 and TNFα but not IFNα whereas pDC (CD11c$^-$ CD123$^+$) respond only to TLR 7 and 9 by producing IFNα and TNF-α but not IL-12 although we could consistently detect 1-2% of IL-12p40 producing pDC upon TLR7/8 triggering. BDCA1$^+$ DC responded to TLR2/6, 3, 4, 5, 7/8 and slightly to TLR1/2 and produced IL-12p40 and TNF-α. In contrast, BDCA3$^+$ DC responsiveness appeared restricted to TLR1/2 (IL-12p40 but not TNF-α) and 3 (IL-12p40 and TNF-α) with a limited responsiveness to TLR9 ligands (IL-12 but not TNF-α). Of note, a higher frequency of BDCA3$^+$ DC produced IL-12p40 as compared to BDCA1$^+$ cells.

Therefore this short and rather simple assay appears suitable to assess the responsiveness of blood DC subsets to TLR ligands in patients.

P4 – Negative Regulation Of Human Osteoclastogenesis by IL-1β And Inhibition of Osteoclastogenesis in Rheumatoid Arthritis Synovial Macrophages

Ji J.D.,[1] Lee B.,[2] Kim T.,[2] Jun J.,[2] Yoo D.,[2] Woo J.,[1] Choi S.J.,[1] Lee Y.H.,[1] Song G.G.,[1] Park-Min K.,[3] Ivashkiv L.B.[3]
1. Division of Rheumatology, College of Medicine, Korea University, Seoul
2. The Hospital for Rheumatic Diseases, Hanyang University, Seoul, Korea

3. Arthritis and Tissue Degeneration Program, Hospital for Special Surgery, New York, NY, USA

Introduction: IL-1β is a key mediator of bone resorption and cartilage destruction in rheumatoid arthritis. IL-1β indirectly stimulates osteoclastogenesis via increased RANKL expression in the stromal/osteoblastic cells, and directly stimulates the resorbing activity of the osteoclasts formed. Recently, we found that TLR ligands inhibit the early steps of human osteoclast differentiation by acting directly on osteoclast precursors. TLRs and IL-1R share a cytosolic domain termed Toll-IL-1R (TIR) domain and common intracellular signaling molecules such as MyD88, IRAK, and TRAF6.
Aims: In this study, we examined the direct effects of IL-1β on osteoclastogenesis in primary human peripheral blood (PB) monocytes and rheumatoid arthritis (RA) synovial macrophages.
Methods: In vitro osteoclastogenesis assays were performed using normal peripheral blood monocytes and RA synovial fluid macrophages. Gene expressions were analyzed using real-time PCR.
Results: IL-1β strongly inhibited human osteoclastogenesis as assessed by generation of $TRAP^+$ multinucleated cells and induced a dramatic decrease in RANK, TREM2 and BLNK mRNA in human PB osteoclast precursors. These inhibitory effects on osteoclastogenesis and expression of osteoclast-related genes were reversed by IL-1 receptor antagonist (IL-1RA).

Primary transcript analysis showed that IL-1β inhibits RANK gene transcription. RANK expression is dependent on M-CSF, and IL-1β inhibited M-CSF induced RANK expression through down-regulation of M-CSF receptor. Similar to normal PB osteoclast precursors, treatment with IL-1β strongly suppressed the osteoclastogenesis and the expressions of RANK, TREM2 and BLNK in RA synovial macrophages. Compared with normal PB osteoclast precursors, osteoclast differentiation from RA synovial macrophages was strongly inhibited.
Conclusion: These results show that IL-1β inhibits osteoclastogenesis by suppression of several osteoclast-related gene expressions in human osteoclast precursors. Inhibition of osteoclast-related genes and M-CSF receptor expression by IL-1β likely serve as a homeostatic mechanism that suppresses excessive bone destruction in inflammatory diseases such as rheumatoid arthritis.

P5 – Effects of Morbid Obesity and Treatment With Ghrelin on T Cell Function

Van der Weerd K.,[1,2] Van Hagen P.M.,[1,2] Dik W.A.,[2] Schrijver B.S.,[2] Kiewiet R.M.,[1] Van Aken M.O.,[3] Van der Lelij A.J.,[1] Van Dongen J.M.,[2] Staal F.J.T.[2]
1. Dept. of Internal Medicine and
2. Dept. of Immunology, Erasmus MC, Rotterdam
3. Dept. of Internal Medicine, HagaZiekenhuis, Den Haag, The Netherlands

Close links exist between metabolism and immunity for example demonstrated by the state of chronic low-grade inflammation in obesity. Many hormones, cytokines, transcription factors and bioactive lipids can function in both metabolic and immune roles. Interestingly, ghrelin, an orexigenic hormone involved in energy balance, and long term regulation of food intake is known to be expressed in the immune system and has immunoregulatory effects. In mice it has been demonstrated that treatment with ghrelin results in increased thymocytes numbers and thymic output. However, until now no human data are available.

Here, we studied the effects of two different forms of ghrelin, unacylated ghrelin (UAG) and acylated ghrelin (AG) on the human immune system in individuals suffering from morbid obesity and controls, focusing on the T cell compartment.

The T cell compartment was defined phenotypically using flow cytometry and T cell receptor excision circle (TREC) analyses. TRECs are circular excision products formed by deleted DNA during T cell receptor gene rearrangements. TRECs are known to have a high over-time stability, but they can not multiply and consequently are diluted during T cell proliferation. Therefore TREC analysis can give information about thymic output and T cell proliferation.

Although treatment with either one of the ghrelin forms had no significant effects on thymic output or T cell proliferation, we did find a highly significant increase ($p<0.001$) in thymic output and T cell proliferation in individuals suffering from morbid obesity compared to lean controls.

These new data suggest that morbid obesity is associated with significant changes in the T cell compartment.

P6 – Identification of Specific Phenotypic Markers for Human Polarized Macrophages

Ambarus C.,[1] Hamann J.,[2] Krausz S.,[1] Tak P.P.,[1] van Eijk M.[3]

1. Clinical Immunology and Rheumatology
2. Experimental Immunology
3. Medical Biochemistry, Academic Medical Center – University of Amsterdam, Amsterdam, The Netherlands

The concept of macrophage polarization describes the heterogeneity of activated macrophages under specific microenvironmental conditions. Based on their pro- or anti-inflammatory functions in mice, two major types of macrophages (M1 and M2) have been described. According to this paradigm, we aim to assess type 2 inflammation in spondyloarthritis (SpA) versus type 1 inflammation in rheumatoid arthritis (RA). Here, we investigated the expression of cell surface molecules on in vitro differentiated human macrophages in order to identify reliable markers for characterization of polarized human macrophages in vivo.

Monocytes were isolated from peripheral blood of healthy donors and patients with RA and SpA and differentiated in vitro for 4 days in the presence of IFN-γ, LPS, TNF-α, IL-4, or IL-10.

In a second set of experiments monocytes were maturated in the presence of GM-CSF or M-CSF and subsequently polarized with Th1 and Th2 cytokines, respectively. Expression of CD14, CD16, CD32, CD64, CD80, CD86, TLR2, TLR4, CD 163, CD200R and CD206 was analyzed by flow cytometry.

M1 macrophages are prototypically induced by IFN-γ (M1a) or by TNF-α or bacterial components like LPS (M1b). In our analysis, IFN-γ induced selective up-regulation of CD64 and CD80 while LPS strongly up-regulated CD14. M2 cells develop in the presence of IL-4 or IL-13 (M2a), immune complexes (M2b), or IL-10, TGF-β, or glucocorticoids (M2c). IL-4 strongly up-regulated CD200R expression whereas IL-10 up-regulated the expression of CD163 and, to a lesser extent, CD16. In contrast to mouse macrophages, CD206 was not a specific marker for IL-4 polarized macrophages in humans. Additionally, CD86 was up-regulated by both IFN--γ and IL-4. TLR2 and TLR4 did not display a specific expression profile. The expression of specific phenotypic markers by polarized cells was similar for monocytes in healthy controls, RA, and SpA. However, the phenotype defined here upon polarization of fresh peripheral blood monocytes only partially holds true when polarizing cells which were already matured with GM-CSF and M-CSF. Especially LPS becomes a potent inducer of both M1 and M2 markers in these conditions. Preliminary data on macrophages isolated from synovial fluid of patients with SpA indicate that different populations of macrophages with distinct phenotypes are present during peripheral arthritis.

Upon polarization in vitro the phenotype of these cells becomes homogenous and the pattern of biomarker expression is similar to that of healthy blood monocyte-derived macrophages.

Our data indicate that CD64 and CD80 are specific surface markers for human in vitro-polarized M1a macrophages and CD14 for the M1b phenotype. CD200R specifically characterizes the M2a and CD163 and CD16 the M2c macrophages. These phenotypic markers allow us to classify polarized human macrophages in inflamed tissue and to study the function and signalling pathways of specific macrophage subsets.

P7 – B Cell Ablative Therapy in Refractory Sight-Threathening Scleritis

van Bilsen K.,[1] Missotten T.,[3] Baarsma G.S.,[3] Kuijpers R.W.,[2] van Laar J.A.M.,[1] van Hagen P.M.[1,3]
1. Internal Medicine
2. Ophthalmology at Erasmus Medical Center, Rotterdam
3. The Rotterdam Eye Hospital, Rotterdam, The Netherlands

Introduction: Scleritis is a rare chronic ocular vasculitis of scleral vessels leading to a substantial amount of morbidity and even blindness. Oral steroids are widely used to treat scleritis and some patients will require intensive immunosuppressive treatment to achieve long-term control of disease. The use of those drugs is limited by adverse effects such as life-threatening infections and malignancies. Moreover, a significant number of patients does not respond.
Aim: We describe two patients with refractory anterior scleritis responding to B cell ablative therapy (rituximab).
Methods: A case series of two patients with therapy refractory anterior scleritis.
Results: Patient 1: A 36-year old female with relapsing polychondritis developed scleritis of the right eye, with photophobia and deteriorating vision. During a period of 18 months she received several immunosuppressives including oral steroids, methotrexate, mycophenolate sodium (MPS), high dose solumedrol, infliximab, cyclophosphamide, and intravenous immunoglobulins (IVIG). Al-

though the other symptoms responded, the scleritis persisted.

This patient received two doses of 1000 mg rituximab with a two-week interval whilst tapering MPS and IVIG. Two months hereafter the scleritis improved and pain resolved. This was accompanied by an significant reduction of peripheral B lymphocyte for at least 6 months.

Patient 2: A 39-year old man was diagnosed with bilateral idiopathic scleritis. Treatment in course of years included high dose steroids, diafenylsulfon, azathioprine, methotrexate, cyclophosphamide and adalimumab. The scleritis only briefly responded and the patient became dependent on high doses of steroids, with unacceptable adverse effects. Ten years after onset of disease, treatment with two doses of 1000 mg rituximab and subcutaneous immunoglobulins was initiated. Within 3 months the inflammation resolved completely and steroids could be stopped. Peripheral B lymphocytes were undetectable for at least 10 months and recovered after 17 months. To date (2 years after rituximab therapy) no relapse occurred.

Conclusions: Clinical activity of scleritis in both patients with severe therapy refractory scleritis disappeared after B cell ablative therapy. Rituximab may be a new promising therapeutic tool in the treatment of refractory scleritis.

P8 – Histone Deacetylase Inhibitors Suppress Inflammatory Cytokine Production by Rheumatoid Arthritis Fibroblast-like Synoviocytes Via inhibition of NF-κB nuclear accumulation

Grabiec A.M., Sanders M.E., Tak P.P., Reedquist K.A.
Div. of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam, The Netherlands

Background and Objectives: The accumulation and persistence of activated fibroblast-like synoviocytes (FLS) contributes significantly to the pathology of rheumatoid arthritis (RA).

Under inflammatory conditions, signaling pathways responsible for cellular activation are tightly regulated by the reversible acetylation and deacetylation of histones, transcription factors and structural proteins. Histone deacetylase (HDAC) inhibitors (HDACi) have demonstrated potent therapeutic effects in animal models of chronic inflammatory disorders.

Aim: The purpose of this study was to examine the effects of HDACi on the activation status of RA FLS and to analyze the molecular mechanism of potential anti-inflammatory effects of HDACi.

Methods: RA FLS were treated with IL-1β or TNFα in the absence or presence of the HDACi trichostatin A (TSA) and suberoyl bis-hydroxamic acid (SBHA). IL-6 and IL-8 production was measured by ELISA. The activity of NF-κB p65 and p50 subunits was measured in FLS nuclear extracts by an ELISA-based activity assay. Nuclear accumulation of p65 and p50 was analyzed by immunoblotting of FLS nuclear fractions. The phosphorylation status of IκBα, and MAP kinases p38 and ERK was assessed by immunoblotting.

Results: Both TSA and SBHA potently and dose-dependently blocked IL-1-induced IL-6 and IL-8 production by RA FLS. 250 nM TSA suppressed production of IL-6 and IL-8 by 60% and 75%, respectively ($p < 0.001$), while 50 μM SBHA reduced the secretion of IL-6 by 30% ($p < 0.05$) and IL-8 by 70% ($p < 0.01$). TSA treatment did not affect the phosphorylation status of IκBα following IL-1 stimulation, but induced a 60% reduction in activity of NF-κB subunits p65 ($p < 0.05$) and p50 ($p < 0.01$) in nuclear fractions of IL-1-stimulated FLS. The suppression of p65 and p50 activity in HDACi-treated FLS was associated with decreased p65 and p50 protein nuclear accumulation. At the same time TSA and SBHA failed to affect the phosphorylation status of p38 and ERK MAP kinases.

Conclusions: We demonstrate that inhibition of HDAC activity in RA FLS efficiently blocks production of inflammatory cytokines IL-6 and IL-8. The inhibition of cytokine production by HDACi is associated with suppression of nuclear accumulation of p65 and p50 NF-κB subunits. Therapies targeting HDAC activity may be useful in suppressing inflammation in RA.

P9 – Vagus Nerve Activity Potentiates TGF-BETA Signaling and Induces Tolerance in Peritoneal and Intestinal Macrophages

de Jonge W.[1], Verseijden C.[1], Boeckxstaens G.[2]
1. Dept. of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, The Netherlands
2. Gastroenterology, KU Leuven, Leuven, Belgium

Background: Vagus nerve efferent stimulation

(VNS) has been shown to ameliorate intestinal inflammation via the peripheral release of acetylcholine. Macrophages express nicotinic acetylcholine receptors (nAChR), and nAChR activation blunts inflammatory cytokine production. However, intestinal macrophages are intrinsically tolerant and produce little cytokines, possibly due to high TGFβ1 levels in the intestinal mucosa. Therefore we investigated whether the anti-inflammatory effect of VNS rests on altered macrophage TGFβ signaling and responses to luminal bacteria.

Methods: To assess the effect of VNS on bacterial uptake in vivo, mice were gavaged 2*10exp7 heat-killed FITC-labelled enterococcus feacum bacteria, after which the right cervical vagus nerve was electrically stimulated for 20min 1 and 5 V at 5Hz frequency.

Bacterial translocation and uptake in the intestinal mucosa was assessed by histological examination 3 hrs thereafter. The effects of nAChR activation on NF-kB activation in a kB luciferase reporter system. Cytokine release, and smad 3, 4 and 7 transcripts were analyzed by ELISA and Light Cycler RT-PCR.

Results: In vivo, VNS led to increased mucosal uptake of orally administered commensal *e.faecium* by phagocytes residing in the intestinal mucosa (0.2+/-0.1 vs 11.2+/-1.2 bacteria/villus in sham vs VNS resp.). Immune-histochemical confocal analysis of the small intestinal mucosa indicated that bacteria were mainly taken up by CD11b+/CD11c-/F4-80+ intestinal macrophages. NAChR beta2/alpha4 was expressed in isolated F4/80+ peritoneal as well as lamina propria macrophages. Activation of this nAChR by nicotine pretreatment (20 min, 1000nM) enhanced phagocytosis of *e.faecium* (2.3-fold;$p<0.05$; compared to control) in lamina propria macrophages. Nicotine reduced the activation of NF-kB and the transcriptional activation of p65 by LPS in a dose-responsive fashion down to 25% of vehicle.

In addition, nicotine (1000nM) reduced expression of the inhibitory smad7 in activated macrophages down to 42% of control, while expression of Smad3 and 4 were not affected.

Conclusions: We conclude that vagus nerve activation potentiates TGFβ signaling in intestinal and peritoneal macrophages, which may contribute to their tolerant phenotype as found in intestinal tissue. This mechanism can explain for the anti-inflammatory effect of VNS in various models of intestinal inflammation.

P10 – Anti IL-17A Therapy Inhibits Tnf-Mediated Bone Loss by Modulation of T Cell Balance

Polzer K.,[1] Koenders M.,[2] Baum W.,[1] Patakas A.,[3] McInnes I.,[4] Joosten L.,[2] Schett G.,[1] van den Berg W.,[2] Zwerina J.[1]

1. Dept. of Internal Medicine 3 and Institute for Clinical Immunology, University of Erlangen-Nuremberg, Erlangen, Germany
2. Dept. of Rheumatology, Radboud University, Nijmegen, The Netherlands
3. Centre for Biophotonics, Strathclyde Institute for Pharmacy and Biomedical Sciences, University of Strathclyde, UK
4. Centre for Rheumatic Diseases, University of Glasgow, Scotland, UK

Objective: Immune activation is the major driver of local and systemic bone loss. Pro-inflammatory cytokines, in particular TNF, link immune activation with bone loss. Recently, T cells have been implicated as regulators of bone turnover. TH1 and TH2 associated lymphokines such as IL-12, IFNγ and IL-4 are strong inhibitors of osteoclastogenesis, whereas IL-17A produced by TH17 cells increases osteoclast differentiation. In this study, we blocked IL-17A in a murine TNF-mediated arthritis model.

Methods: Human TNF transgenic mice were treated with an anti-IL-17A antibody for 4 weeks. Mice were clinically and histologically assessed for signs of inflammation, cartilage damage and bone damage. T-cell balance was evaluated with quantitative mRNA analysis of T-cell associated genes and measurement of serum cytokines. Moreover, we analysed the effects of IL-17A blockade in hTNFtg mice devoid of IL-1 signalling.

Results: Despite only minor effects on inflammation IL-17A blockade effectively reduced local and systemic bone loss based on reduced osteoclast differentiation in vivo. These effects were due to a shift to bone- protective T-cell responses including TH2 differentiation, induced IL-4 and IL-12 expression and increase in foxp3-expressing lymphocytes. When blocking IL-17A in IL-$1^{-/-}$TNFtg mice, arthritis was virtually abrogated and no osteoclasts and bone erosions formed. Moreover, no shift in T cell lineages was observed in IL-$1^{-/-}$TNFtg mice treated with IL-17A and no additional benefit of IL-17A blockade on bone mass was found.

Conclusion: We thus conclude that IL-17A regulates bone mass in conjunction with IL-1 through

suppression of bone regulatory pathways of T cell mediated adaptive immunity.

P11 – Investigation of the Migratory and Pathogenic Properties of Bystander T Cells *In Vitro* and *In Vivo*

Bryant J., Ahern D.J., Brennan F.M.
Kennedy Institute of Rheumatology, Imperial College London, UK

Introduction: Bystander lymphocytes generated *in vitro* by cytokine stimulation (IL-2, IL-6, TNFα) were previously shown to induce TNFα production from monocytes in a contact-dependent manner. These cytokine activated T cells (Tck) resemble T cells isolated from rheumatoid arthritis synovial tissue in phenotype and by the signalling pathways they activate in monocytes. The Tck are therefore used as a surrogate model for studying the RA T cell.
Aim: The aim of this project is to extend the phenotypic data and effector function studies of the Tck and investigate whether this data reflects a functional migratory and pathogenic potential *in vitro* and *in vivo*.
Methods: Lymphocytes were obtained by elutriation and CD4 population enriched using positive selection. HUVECs were isolated from umbilical cords of consented patients undergoing scheduled C sections. Cell phenotype was determined by flow cytometry. *In vitro* chemotaxis and transendothelial migration assays determined the migratory potential of the Tck. Tck effector function was explored using co-culture with monocytes or endothelial cells followed by ELISA analysis.
Results: Phenotypic data showed an upregulation of CXCR3, CXCR4, CCR5 and CCR6 on the Tck compared to matched resting T cells. However the chemotaxis assays showed functional migration of Tck only to SDF-1, ligand of CXCR4. Transendothelial migration assays showed that Tck spontaneous and stimulated migration was significantly higher than matched resting or antiCD3/CD28 stimulated T cells. Use of blocking antibodies indicated a role for CD29, CD49d, CD11a, CD18 and CXCR4 in this process. Studies to further explore this migration, the effector function of the migrated cells and microarray analysis of the HUVECs are ongoing.
Conclusion: The inflammatory phenotype of the Tck results in higher transendothelial migration compared to resting or antigen activated T cells; this role *in vivo* is currently being explored using the RA/SCID mouse model.

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P12 – The PEG Component of Certolizumab Pegol Inhibits Degranulation by Stimulated Mast Cells

Bracher M., Lamour S., Nesbitt A.
UCB, Inflammation Discovery, Slough, UK

Introduction: The administration of some conventional injectable TNF inhibitors is associated with severe injection-site pain (ISP). ISP may be linked to the inflammatory mediators released upon mast cell degranulation. In contrast to conventional TNF inhibitors, certolizumab pegol (CZP) lacks an Fc region, and consists of a Fab' linked to a 40 kDa PEG moiety. A low incidence of ISP has been observed in patients receiving CZP in clinical trials in patients with rheumatoid arthritis and Crohn's disease (CD).
Aim: To determine if the PEG moiety of CZP inhibits non-immune-stimulated mast cell degranulation and may be responsible for the low incidence of ISP with CZP.
Methods: Mast cells were cultured in vitro from stem cells over an 8–12 week period using the method of Saito et al (1). Mast cell degranulation, as measured by β hexosaminidase release, was stimulated by addition of compound 48/80. Titrations of CZP, PEG, and a mixture of PEG and naked Fab' at a PEG concentration of 45 mg/mL were incubated with mast cells and a fixed amount of compound 48/80 to determine the effect on mast cell degranulation. Mast cell viability was assessed using the Promega Cell titer 96 Aqueous One Solution cell proliferation assay.
Results: Compound 48/80 stimulated mast cell degranulation although the absolute level varied between cell preparations. PEG (45 mg/mL) inhibited mast cell degranulation stimulated by 20μM and 200μM compound 48/80 by 66% (n=3) and 57.5% (n=4) respectively ($P < 0.001$). CZP (100 mg/mL), PEG alone (45 mg/mL) and the mixture of PEG (19.8 mg/mL) and naked Fab' (23.9mg/mL) all

inhibited the majority of mast cell degranulation. None of the reagents affected overall cell viability.
Conclusion: PEG inhibited compound 48/80–stimulated degranulation of mast cells. The concentrations at which an effect is observed are what might be expected at the injection site but not systemically. This beneficial effect of PEG on mast cells may explain the low level of ISP observed with CZP in clinical trials in RA and CD. However, the exact mechanism behind this activity is unclear and warrants further investigation.

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P13 – Platelet-Derived Growth Factor-BB: a Stimulus for Cytokine Production by Orbital Fibroblasts in Graves' Ophthalmopathy

van Steensel L.,[1] Paridaens D.,[2] Dingjan G.M.,[1] van Hagen P.M.,[1] Hooijkaas H.,[1] Dik W.A.[1]
1. Dept. of Immunology, Erasmus MC, Rotterdam
2. Rotterdam Eye Hospital, Rotterdam, The Netherlands

Introduction: Graves' ophthalmopathy (GO) is characterized by infiltration of immune cells into the orbit, a process in which cytokines play a central role. Orbital fibroblasts are believed to play an important role in GO and are potent producers of cytokines upon different stimuli.
Recently, we showed increased expression of the PDGF-B chain in GO orbital tissue and a stimulatory effect of the dimeric PDGF-BB molecule on fibrosis in GO. PDGF-BB has also been described to activate the NF-kB pathway, which is well recognized for its role in regulating cytokine production.
Aim: The current study was conducted to determine whether PDGF-BB induced orbital fibroblasts to produce pro-inflammatory cytokines associated with GO and whether the NF-kB signaling is involved herein. In addition we tested whether orbital fibroblasts were unique in their reaction upon PDGF-BB stimulation compared to fibroblasts from other anatomical locations.
Methods: Orbital, lung and skin fibroblasts were stimulated with PDGF-BB and IL-1β, IL-6, IL-8, IL-16, CCL2, CCL5, CCL7 and TNF-α production was determined by ELISA.

Involvement of NF-κB activation through PDGF-signaling was investigated by Electrophoretic Mobility Shift Assay (EMSA), specific NF-κB inhibitors and the PDGF-receptor kinase inhibitor imatinib mesylate.
Results: IL-6, IL-8, CCL2, CCL5 and CCL7 production by orbital fibroblasts was enhanced by PDGF-BB stimulation while IL-16, IL-1ß and TNF-α production was not affected. PDGF-BB induced NF-κB activity in orbital fibroblasts and both NF-κB inhibitors and imatinib mesylate reduced PDGF-BB induced cytokine production. Furthermore, similar, but less vigorous effects of PDGF-BB on cytokine production were observed in lung and skin fibroblasts.
Conclusion: PDGF-BB induces production of pro-inflammatory cytokines via the NF-kB pathway and orbital fibroblasts are special in that they excel in this function over fibroblasts from other anatomical locations. These data imply a role for PDGF-BB in regulating orbital inflammation in GO and identifies the PDGF-signaling cascade as a therapeutic target in GO.

P14 – Decreased Glucocorticoid Sensitivity in Early Arthritis and Established Rheumatoid Arthritis

Quax R.A.M.,[1] Koper J.W.,[1] Weel A.E.,[2] Huisman M.A.,[3] Luime J.J.,[4] van Zeben D.,[3] de Jong P.H.P.,[4] de Jong F.H.,[1] Lamberts S.W.J.,[1] Hazes J.M.W.,[4] Feelders R.A.[1]
1. Dept. of Internal Medicine, Erasmus MC, University Medical Center, Rotterdam
2. Dept. of Rheumatology, Maasstad Hospital, Rotterdam
3. Dept. of Rheumatology, Sint Franciscus Gasthuis, Rotterdam
4. Rheumatology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Introduction: Apart from a blunted hypothalamic-pituitary-adrenal axis activity, alterations in glucocorticoid (GC) sensitivity might play a role in the susceptibility to develop rheumatoid arthritis (RA). GCs exert their effects by transrepression or transactivation of target genes.

We have assessed GC sensitivity of peripheral blood mononuclear cells (PBMC) using a bioassay in patients with early arthritis and established RA.
Patients: A multicenter stratified randomized single-blind controlled trial is currently being performed in patients 18 years or older with recent-onset arthritis (tREACH-study)[3].

Patients with a high probability (>70%) of persistent arthritis were selected according to a prediction model (N=41). In an independent cohort of

patients with established RA and active disease (FLARE-study), additional patients were recruited (N=16). Patients in the tREACH were treatment-naïve. Patients in the FLARE-study were not treated with GC for at least 3 months.

Methods: PBMC were obtained from all patients and healthy controls. Cellular GC-sensitivity was determined *in vitro* using a bioassay measuring GC-specific transactivation of the GC-induced leucine zipper (GILZ) and transrepression of the interleukin-2 (IL-2) gene using qRT-PCR. Half maximal effective concentration (EC_{50}) was used as a read-out for *in vitro* GC-sensitivity. Differences between the different groups were tested by analysis of variance (ANOVA).

Results: IL2-EC_{50} values (mean ± SD) were higher in patients with RA (8.43 nM ± 0.39, $p<0.001$) and patients with recent-onset arthritis and high chance of developing persistent arthritis (8.82 nM ± 1.72, $p<0.001$) when compared to healthy individuals (3.48 nM ± 0.71).

Patients with low (DAS44 <2.4), moderate (DAS44≥2.4 and <3.7) and high disease activity (DAS44≥3.7) had similar IL2-EC_{50} values. GILZ-EC_{50} values did not differ between patients and healthy controls.

Conclusions: Patients with established RA had a decreased *in vitro* cellular GC-sensitivity at the level of GC-regulated gene transcription as measured by the IL-2 bioassay.

Interestingly, also in patients with recent-onset arthritis (<1 yr) a diminished GC-sensitivity was observed. This might imply that an altered GC-sensitivity plays a role in the pathophysiology of RA. Although (severe) inflammation has been reported to influence GC-sensitivity we did not find differences in GC-sensitivity when patients were stratified according to their disease activity.

P15 – The Cartilage-Specific Molecule, Melanoma Inhibitory Activity Protein, Contributes to Collagen-Induced Arthritis

Baeten D.L.P.,[1] Bosserhoff A.,[2] Cantaert T.,[1] Tak P.P.,[1] Yeremenko N.[1]

1. Division of Clinical Immunology and Rheumatology, Academic Medical Centre/University of Amsterdam, Amsterdam, The Netherlands

2. Institute of Pathology, University of Regensburg, Regensburg, Germany

Introduction: Melanoma inhibitory activity (MIA) is a small chondrocyte-specific protein with unknown function, which is regulated similarly as collagen type II [1]. MIA-deficient mice ($MIA^{-/-}$) have a normal phenotype and only minor micro-architectural alterations of the cartilage [2]. We recently demonstrated that the immunodominant epitopes of MIA are actively presented in an HLA-DR4-restricted manner in the inflamed rheumatoid arthritis (RA) joint [3].

Aim: To assess the potential role of MIA as autoantigen, we investigated here collagen-induced arthritis (CIA) in wild type C57BL/6 (WT) and $MIA^{-/-}$ mice.

Materials and Methods: 12 weeks old wild type C57BL/6 (N=33) and $MIA^{-/-}$ (N=24) female mice were immunized intradermally at the base of the tail with chicken type II collagen emulsified in an equal volume of complete Freund's adjuvant; this procedure was repeated as a boost 21 days later. The severity of the arthritis was assessed using a semi-quantitative scoring system (0 to 4). For flow cytometric analysis splenocytes were collected at day 10 after primary immunization and restimulated in vitro with anti-CD3/CD28, collagen type II or MIA.

Results: $MIA^{-/-}$ mice developed markedly reduced *incidence of arthritis* compared to WT mice *(50% versus 87.9%), which resulted in* a significantly lower clinical arthritis score.

Phenotyping of splenocytes showed higher percentages of B cells ($p<0.001$) and $CD4^+$ T cells ($p<0.0001$) in $MIA^{-/-}$ mice. Furthermore MIA-deficient mice had fewer $CD8^+$ T cells ($p<0.0001$) in their spleen than WT animals. Ex vivo restimulation of isolated after primary immunization lymphocytes with MIA protein showed a significantly increased proliferation in WT versus $MIA^{-/-}$ mice. Accordingly, there was a significant increase of FoxP3-expressing $CD25^+CD4^+$ regulatory T cells ($p<0.05$) upon restimulation with MIA protein in $MIA^{-/-}$ mice.

Conclusion: MIA contributes to collagen-induced arthritis. MIA-deficient mice are partially protected against CIA. The inhibition of lymphocyte proliferation and increase of regulatory T cells upon restimulation with MIA *in vitro* support a role for MIA as autoantigen. Ongoing analyses are investigating how this translates into bone and cartilage damage in these mice.

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P16 – Hemorheological Changes Associated with Systemic Lupus Erythematosus and Rheumatoid Arthritis

Santos M.J.,[1,2] Vinagre F.,[2] Capela S.,[3] Canhão H.,[1,3] Canas da Silva J.,[2] Saldanha C.,[4] Fonseca J.E.[1,3]
1. Rheumatology Research Unit, Instituto Medicina Molecular da Faculdade de Medicina de Lisboa
2. Rheumatology Dept., Hospital Garcia de Orta, Almada
3. Rheumatology and Bone Metabolic Diseases, Hospital Santa Maria, Lisboa
4. Biochemistry, Faculdade de Medicina de Lisboa, Portugal

Introduction: The increased cardiovascular (CV) risk associated with inflammatory diseases such as Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA) still remains to be fully explained. Hemorheological changes have been associated with myocardial infarction and stroke in the general population. Although hemorheological parameters are altered in inflammatory states and may add to CV risk, data concerning SLE and RA patients is scarce.
Aim: To evaluate hemorheological parameters in SLE and RA and to determine the effect of disease activity in these parameters.
Patients and Methods: Whole blood viscosity, erythrocyte deformability at low and high shear rates, plasma viscosity, erythrocyte aggregation, plasma fibrinogen and hematocrit were determined in women with SLE and RA, without previous CV events and compared with healthy controls. Disease activity, as well as the presence of comorbidities and medication was assessed.
Results: One hundred and sixty six women, aged 48.7 years (SD -13.6) were evaluated (64 with SLE, 57 with RA and 45 controls). Hypertension was more common among SLE and RA patients, but otherwise the groups were well balanced for the presence of obesity, diabetes, dislipidemia, and smoking. A significant decrease in erythrocyte deformability in the shear stress range 0.6-6 Pa was found in SLE and RA patients compared to healthy controls.

Conversely, erythrocyte aggregation measured at 5 and 10 seconds was significantly higher in SLE and RA. The difference remained significant after adjusting for hematocrit, age, corticosteroid use and comorbidities. No significant differences in other rheological parameters were observed. Although we could not depict a significant relation between hemorheological changes and disease activity, a trend toward lower red cell deformability in active SLE (39.2% in patients with SLEDAI>4 vs 41.2% in those with SLEDAI ≤ 4) was detected.
Conclusion: SLE and RA patients present some alterations in hemorheological parameters, which are more pronounced in lupus patients. Taking into consideration that changes in erythrocyte deformability and aggregation compromise the ability of red blood cells to pass through capillaries and impair tissue oxygen supply, the abnormalities found in SLE and RA may be clinically relevant and may contribute to circulatory disorders in these patients.

	SLE n=64	RA n=57	Control n=45
Deformability % 0.6 Pa	4.73 (2.1)*	4.38 (1.96)#	5.61 (1.92)
Deformability % 6 Pa	40.8 (4.6)*	41.4 (4.2)*	42.8 (3.7)
Aggregation 5'	11.9 (2.4)§	11.9 (2.3)*	10.8 (1.9)
Aggregation 10'	18.6 (3.9)§	18.5 (3.9)§	16.8 (2.9)
Aggreg. adjusted for Hct 45%	19.7 (4.2)§	19.4 (4.1)*	17.8 (3.4)

Differences statistically significant versus control group *p=0.02; #p=0.002; §p=0.01

P17 – Anti DKK-1 Treatment Protects Tnf-Transgenic Mice From Inflammatory Osteoporosis

Ruiz G.,[1] Polzer K.,[1] Baum W.,[1] Axmann R.,[1] Krönke G.,[1] Schett G.,[1] Zwerina J.[1]
1. Dept. of Internal Medicine 3, University of Erlangen-Nuernberg, Germany

Introduction: Chronic inflammation is a major risk factor for systemic bone loss leading to osteoporotic fracture and substantial risk of morbidity and mortality. Inflammatory cytokines are thought to play a key role in the pathogenesis of inflammation and the Wnt signalling pathway was shown to regulate joint destruction in animal models of arthritis.
Study Aim: To analyze whether stimulation of the Wnt pathway with an anti-DKK1 antibody alters inflammatory osteopenia in an animal model of arthritis.
Methods: Six weeks old human TNF transgenic (hTNFtg) mice were treated with the following agent during four weeks: Vehicle (PBS), an anti-TNF antibody (10 mg/kg three times a week), a rat antibody to mouse DKK-1 (10 or 30 mg/kg three times a week) or a combination of anti-TNF and anti-DKK-1. Systemic bone mineral density was analyzed by micro computed tomography and

bone histomorphometry. Calcein labelling was used for dynamic histomorphometry. In vitro, isolated osteoblasts were stimulated with TNF and analyzed functionally.

Results: Treatment with anti-TNF partially reversed systemic bone loss. Despite leaving synovial inflammation untouched, anti-DKK1 treatment dose dependently inhibited TNF-induced bone loss. The combination of anti-TNF and anti-DKK1 was the most efficient treatment strategy. In contrast to anti-TNF, anti-DKK1 treatment leads not only to a decrease in osteoclast numbers but also an increase in bone formation, having a protective effect on bone morphology. In the present study, we could demonstrate the importance of DKK-1 as regulator of bone metabolism in TNF induced osteopenia.

Conclusions: These data indicate that modulation of the Wnt pathway essentially influences TNF-mediated bone loss. Despite TNF-mediated inflammatory arthritis, systemic bone is protected by treatment with an anti-DKK1 antibody.

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P18 – TLR2 Promotes TH2/TH17 Responses Via TLR4 and TLR8 by Human Dendritic Cells by Abrogating The Type 1 Interferon Amplification Loop

Broen J.,[1] Lubberts E.,[2] Radstake T.,[1] Santegoets K.,[1] van Bon L.,[1] van den Berg W.,[1] van Riel P.,[1] Wenink M. [1]

1. Dept. of Rheumatic Diseases, Radboud University Nijmegen Medical Center, Nijmegen;
2. Dept. of Rheumatology, Erasmus Medical Center, Rotterdam, The Netherlands

Background: While the function of most Toll-like Receptors (TLRs) seems clearly aimed at removing invading bacteria and viruses the precise function of TLR2 is less clear.

Aim: We aimed to delineate the role for TLR2 in the modification of the phenotype and functionality of human Dendritic Cells (DCs) and its interaction with other TLRs.

Patients and Methods: Monocyte-derived DCs were cultured from 15 healthy controls and in vitro experiments were performed to investigate the effect of TLR2 activation on other TLRs.

Results: Both P3C (TLR2/1) and P2C (TLR2/6) dose-dependently inhibited LPS (TLR4) and R848 (TLR7/8) induced cytokine production, particularly IL-12p70, but not Flagellin (TLR5) and even enhanced Poly(I:C) (TLR3) mediated cytokine production. This was not due to hindrance of the binding of the TLR ligands as was shown by using stably transfected HEK-TLR4 cells. TLR2 inhibits the type 1 interferon (IFN) amplification loop of TLR4 and 7/8, which is crucial for the induction of IL-12p70, by abrogating the production of type 1 IFNs, inhibiting the phosphorylation of STAT1 and strongly reducing the transcription of IRF1 and IRF8. In line with this, an increased expression of SOCS1 was observed. Furthermore, the inhibitory effect of TLR2 on the release of TNFα but not of IL-12p70 was mediated by PI3K.

Erk or IL-10 did not play a role in the suppression by TLR2. In MLR experiments TLR2 co-activation of the DCs in addition to stimulation with LPS and R848 led to a significant shift from Th1 to Th2 and Th17 prone responses compared to the stimulation with LPS and R848 alone. This was shown to be due to the inability of TLR2 co-stimulated DCs to produce IL-12p70. We found that IL-6 and IL-1 were essential in DC facilitated Th17 differentiation.

Conclusions: Here, we report that TLR2 is able to dampen TLR4 and 7/8 induced cytokine production by DCs which led to a shift from Th1 to Th2 and Th17 cell differentiation. This puts TLR2 in the middle of the immune network deciding whether the effector response against microorganisms or in autoimmunity is mainly Th1 or Th17 mediated.

P19 – The Inhibitory FCIIB Receptor Dampens Toll-like Receptor 4 Mediated Immune Responses and is Selectively Up Regulated on Dendritic Cells from Rheumatoid Arthritis Patients with Quiescent Disease

Bonvini E.,[1] Koenig S.,[1] Radstake T.,[2] Santegoets K.,[2] van den Berg W.,[2] van Riel P.,[2] Wenink M.[2]

1. Macrogenics Inc, Rockville, USA
2. Dept. of Rheumatic Diseases Radboud University Nijmegen Medical Center Nijmegen, The Netherlands

Introduction: Despite extensive research little is known about the exact pathogenesis of Rheumatoid Arthritis (RA), let alone the mechanisms at work aimed at dampening the chronic inflammatory response.

Aim: In the present study we aimed to study whether RA patients who are able to successfully discontinue anti-rheumatic treatment have phenotypically and functionally different dendritic cells (DCs) compared to healthy controls and RA patients in need of anti-rheumatics.
Patients and Methods: All patients in our well documented cohort who were not on DMARD therapy for more than 2 years were selected (DMARD(-) RA, N=16) and matched with RA patients in need of DMARD therapy (DMARD(+) RA, N=16). Additionally ten healthy controls were included. Monocytes were isolated and cultured into monocyte-derived DCs and in vitro experiments were performed.
Results: DCs from RA patients able to successfully halt DMARD therapy expressed very high levels of the inhibitory FcγRIIb compared to RA patients on DMARDs or healthy controls. All other markers were equally expressed on DCs from DMARD(-) RA and DMARD(+) RA patients and healthy controls. The expression of FcγRIIb on DCs was negatively correlated with disease activity in DMARD(-) RA patients. Only DCs from DMARD(-) RA patients were able to inhibit pro-inflammatory TLR4 responses when co-stimulated with ICs, lowering the release of TNFα and IL-12p70 and dampening the ability to induce T cell proliferation. In addition the production of Th2 cytokines by T cells as well as the presence of regulatory T cells was markedly enhanced. By the use of blocking antibodies we demonstrate that FcγRIIb is crucial for the inhibitory effect of ICs on TLR4 responses. The inhibitory effect of FcγRIIb is mediated via the PI_3K/Akt pathway and is characterized by the increased phosphorylation of SHIP and Akt and the decreased degradation of IkBα.
Conclusion: Our data collectively indicate that FcγRIIb is decisive in the modulation of the TLR4 mediated immune response, which might be crucial in generating sustained low RA disease activity.

P20 – Generation of ANTI-NAG-2 Monoclonal Antibody from Patients' Memory B Cells: Implications for a Novel Therapeutic Strategy in Systemic Sclerosis

Traggiai E.,[1*] Lunardi C.,[2*] Bason C.,[2] Dolcino M.,[1] Corrocher R.,[2] Puccetti A.[1,3]
1. Dept. of Clinical and Experimental Immunology, Institute G. Gaslini, Genova
2. Dept. of Clinical and Experimental Medicine, University of Verona, Policlinico G.B. Rossi, Verona
3. Dept. of Experimental Medicine, University of Genova, Genova, Italy
*These authors contributed equally to this study

Aim: We have previously reported that antibodies directed against the Cytomegalovirus-derived protein UL94 cross-react with the cell surface tetraspanin NAG-2 molecule inducing apoptosis of endothelial cells and activation of fibroblasts in patients with systemic sclerosis (SSc). We aimed at generating a non functional monoclonal antibody directed against NAG-2 from patients' memory B cells.
Methods: Direct and competitive ELISA methods have been used to evaluate the binding of antibodies from scleroderma patients' and controls' sera to the NAG-2 peptide.

Immunoglobulin G memory B cells were sorted, EBV transformed and cloned to obtain NAG-2 specific monoclonal antibodies. Endothelial cells and fibroblasts were cultured under standard conditions and used for functional assays.
Results: Anti-NAG-2 purified antibodies obtained from patients' Immunoglobulins induce endothelial cells apoptosis and fibroblast proliferation. Patients' Immunoglobulins depleted of the anti-NAG-2 fraction do not exert such functional activity. Therefore the NAG-2 molecule represents a potential novel candidate for therapeutic intervention in SSc. Here we describe the generation of a human monoclonal antibody directed against the NAG-2 molecule. Such monoclonal antibody does not retain any functional property and is able to block the effect of serum pathogenetic anti-NAG-2 antibodies.
Conclusions: The majority of SSc patients present antibodies directed against tetraspanin NAG-2 and mediate both endothelial cells apoptosis and fibroblast proliferation, features of the disease. The anti-NAG-2 human monoclonal antibody we have obtained blocks signal transduction and therefore may be a potential candidate for a new treatment in SSc, a disease where the current biological therapies have little or no efficacy.

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P21 – Cytokine Network in the First 6 Weeks of Rheumatoid Arthritis Onset

Cascão R.,[1*] Moura R.A.,[1*] Canhão H.,[1,2] Sousa E.,[1,2] Mourão A.F.,[1,3] Rodrigues A.M.,[1,2] Polido-Pereira J.,[1,2] Viana Queiroz M.,[1,2] Rosário H.S.,[4] Souto-Carneiro M.M.,[5] Graça L.,[6] Fonseca J.E.[1,2]

1. Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisboa
2. Rheumatology Dept., Hospital de Santa Maria, Lisboa
3. Rheumatology Dept., Hospital Egas Moniz, Lisboa
4. Microvascular Biology and Inflammation Unit, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisboa
5. Center for Neurosciences and Cell Biology, Autoimmunity Group, Coimbra
6. Cellular Immunology Unit Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

*These authors contributed equally to this work

Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease mainly characterized by synovial hyperplasia and joint destruction. Although the etiopathology of this autoimmune disease is not completely understood, it is known that it is associated with a misregulation of both the cellular immune system and the cytokine network. Nevertheless, little is known about the blood cytokine milieu in the first few weeks of RA.
Aim: The objective of this study was to determine cytokines' concentration in blood (serum) samples from patients with undiagnosed polyarthritis with less than 6 weeks of duration that later on evolved into RA (very early RA, VERA) or into other conditions (non-RA, VEA).
Patients and Methods: Levels of a panel of cytokines and chemokines (IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 (p70), IL-17A, IL-18, IL-22, IL-23, IFN-γ, Leptin, MCP-1, MIP-1α, OPG, TGF-β and TNF) were measured in serum samples of patients and controls by FlowCytomix assay kit. IL-21, APRIL and BAFF levels were determined by ELISA**.**
Results: VERA patients have increased levels of IL-1β, IL-6, IL-8, IL-17A, IL-22, APRIL, MCP-1 and Leptin at baseline in comparison with healthy controls. Interestingly, IL-1β, IL-8, Leptin and BAFF were higher in VERA as compared to VEA patients. Both VERA and VEA patients have reduced levels of TGF-β when compared to controls. No statistically significant differences could be observed for IL-4, IL-12, IL-18, MIP-1α or OPG. Regarding IL-2, IL-10, IL-23, IFN-γ and TNF the level of production was undetectable or very low in all early arthritis patients.
Conclusion: In very early arthritis the increased production of serum IL-1β, IL-8, IL-17A and MCP-1 could support neutrophil's and macrophage's activation and migration towards the synovium. The elevated IL-6 and APRIL levels generated during the inflammatory process could stimulate and activate B cells towards the synovial membrane. Furthermore, the increase of IL-1β and IL-6 might be related to the early driving of Th17 differentiation observed in these patients. These observations might be of interest for targeting selection of very early arthritis patients.

P22 – Clinical and Diagnostic Value of Ribosomal P Autoantibodies in Systemic Lupus Erythematosus

Bahloul Z.,[1] Fourati H.,[2] Hachicha J.,[3] Haddouk S.,[2] Marzouk S.,[1] Masmoudi H.,[2] Samoud S.[4]

1. Dept. of Medicine, Hedi Chaker University Hospital, Sfax
2. Dept. of Immunology, Habib Bourguiba University Hospital, Sfax
3. Dept. of Nephrology, Hedi Chaker University Hospital, Sfax
4. Dept. of Immunology, Farhat Hache University Hospital, Sousse, Tunisia

Introduction: Although antibodies to ribosomal P antibodies have only been recognized since 1985, many recent research developments have served o highlight the special significance of these autoantibodies.
Aim: To analyse prospectively the diagnostic sen-

sitivity and specificity, as well as the clinical relevance of ribosomal P autoantibodies (anti-P) in a large cohort of systemic lupus erythematosus (SLE) patients.

Patients and Methods: Anti-P were evaluated in the serum of 200 Tunisian SLE patients at disease onset and 130 various control subjects by a sensitive immunodot assay. A complete laboratory evaluation and clinical examination were performed in each SLE patient. During the follow-up, the patients were regularly monitored for clinical parameters. Global SLE activity was measured by the European Consensus Lupus Activity Measure (ECLAM).

Results: The sensitivity and specificity of anti-P testing for SLE were 23.5 % and 98.4%, respectively. 14/47 (29.8 %), 27/47 (57.4 %), and 5/47 (10.6 %) anti-P positive samples were negative for anti-dsDNA, anti-Sm, or both antibodies, respectively.

The anti-P positive patients showed more active disease activity and a much higher prevalence of arthritis. An association between IgG anticardiolipin antibodies and anti-P was also found. However, anti-P were not associated with neuropsychiatric manifestations or lupus nephritis.

Conclusion: This study does not seem to confirm the described association of anti-P with neuropsychiatric manifestations of SLE. However, it supports the anti-P association with arthritis and disease activity as well as the presence of anticardiolipin antibodies.

Based on our study and other related studies, we propose that, akin to anti-Sm and antidsDNA, anti-P antibodies detected by one agreed method may be considered for inclusion as a criterion for the classification of SLE.

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P23 – Dendritic Cell Changes in RA Synovial Tissue after Infliximab Treatment

Lebre M.C., Oliveira A.S.F., Ramos M.I., Wijbrandts C.A., Aarrass S., Tak P.P.

Div. Clinical Immunology/Rheumatology, AMC/ /University of Amsterdam, Amsterdam, The Netherlands

Purpose: Tumor necrosis factor-alpha (TNF-α) blockade using infliximab, a chimeric anti-TNF-α antibody, is an effective treatment for psoriatic arthritis (PsA) and rheumatoid arthritis (RA). It has previously been shown that infliximab reduces the synovial infiltrate very early after initiation of treatment. Dendritic cell (DC) subsets are critically involved in RA pathogenesis. It is at present unknown whether anti-TNF therapy exerts its effects in part via an effect on synovial DC subsets, taking into account that myeloid DC (mDC) might have a more inflammatory role in RA compared to plasmacytoid DC (pDC). Therefore, we investigated whether infliximab treatment changes the numbers of synovial DC subsets (mDC and pDC).

Methods: Thirteen patients with active RA were randomized to received either a single infusion of infliximab (3 mg/kg) (n=7) or placebo (n=6) intravenously. All patients were subjected to an arthroscopic synovial biopsy immediately before initiation, and 48 hour as well as 28 days after initiation of treatment. Immunohistochemical analysis was performed to analyze the inflammatory infiltrate (CD3 and CD68) and $CD1c^+$ (mDC and $CD304^+$ pDC.

Stained tissue sections were quantified by digital image analysis.

Results: There was a highly significant reduction of synovial $CD1c^+$ mDC 28 days after initiation of infliximab treatment (from [mean±SEM] 6782±3304

cells/mm^2 to 602±141 cells/mm^2, P=0.0047). We found already a clear trend 48h after infusion (1469±426 cells/mm^2, P=0.1375). In contrast, there were no statistically significant changes in the numbers of CD304$^+$ synovial pDC after infliximab treatment (at baseline: 4609±1413 cells/mm^2, at 48h: 3687±426 cells/mm^2, at 28d: 1787±494 cells/mm^2; P=0.5338 and P=0.1014, respectively).
Conclusions: These findings suggest that infliximab treatment exerts a prominent effect on the findings suggest more inflammatory mDC subset compared to pDC, resulting in immunomodulation and resolution of inflammation.

P24 – Splenic DC Subsets During Collagen-induced Arthritis in Mice: a Role for Inflammatory Conventional DC?

Lebre M.C.,[1] Bevaart L.,[2] Ramos M.I.,[1] Aarrass S.,[1] Koepke J.,[2] Tak P.P.[1]
1. Div. of Clinical Immunology/Rheumatology, AMC/University of Amsterdam, Amsterdam
2. Arthrogen BV, Amsterdam, The Netherlands

Purpose: Dendritic cells (DC) play a pivotal role in the orchestration of T cell immunity and tolerance due to their ability to stimulate naive T cells and direct effector cell function.

Immunomodulated and tolerogenic DC could be used to ameliorate arthritis. Therefore, in order to gain insight into the characteristics of DC subsets in murine collagen-induced arthritis (CIA) we analyzed the frequencies and phenotype of conventional (c)DC and plasmacytoid (p)DC in a murine C57Bl/6 CIA model during different stages of the arthritis process.
Methods: Arthritis was induced in female C57Bl/6 mice on day 0 (i.d. injection of a chicken collagen/CFA emulsion at the base of the tail) and on day 21 i.d. injection with this emulsion was repeated. Mice were inspected daily from day 20 on for signs of arthritis by two independent observers. Clinical scores were assigned using an established method. At different time points (at days 20, 30, 41 and 63 after CIA induction), mice were sacrificed and spleens were collected. The frequency and phenotype of cDC and pDC was assessed by FACS using specific antibodies: CD11c (total cDC), CD8α (to distinguish 2 cDC populations: CD8α$^+$ and CD8α$^-$) and PDCA-1 together with B220 and Ly-6C (pDC). In addition, isolated cDC and pDC were stimulated for 48h with LPS or CpG, respectively, and cell-free supernatants were analyzed for the contents of inflammatory cytokines using a cytokine-bead assay (CBA).
Results: As expected clinical signs of arthritis started at day 22 (mean±SEM, 0.125±0.125). On days 30, 41 and 63 the mean clinical scores were 1.000±0.555, 2.563±0.922 and 6.500±0.945 respectively. At all the time points studied, the frequencies of splenic cDC (total CD11c$^+$, CD8α$^+$ or CD8α) exceeded significantly those of pDC (PDCA-1$^+$B220$^+$Ly-6C$^+$) except CD8α$^-$ at days 20 and 30. Within CIA mice the frequencies of CD8α$^-$ increased significantly (compared to day 20) starting from day 30 while the frequencies of CD8α$^+$ and pDC were significantly increased on day 63 only. When all the DC subsets from CIA mice were compared to those present in mice without CIA the frequencies of total CD11c, CD8α$^+$, CD8α$^-$ and pDC were significantly increased on day 63. Interestingly, within CIA mice activated cDC produced significantly higher levels of IL-6 on days 41 and 63 while TNF-α was increased only on day 63 (compared to day 20). In contrast, IL-6 and TNF-α-derived from pDC decreased on day 63.
Conclusions: The observation that cDC subsets and their inflammatory cytokines are significantly increased during the development of CIA and that pDC-derived inflammatory cytokines are decreased suggests an inflammatory role for cDC and an regulatory role for pDC in the arthritic process. Thus, cDC rather than pDC may represent an important therapeutic target in arthritis.

P25 – Modulating TLR Responses in Systemic Sclerosis Via Heme Oxygenase-1

van Bon L.,[1] Scharstuhl A.,[2] Pennings B.C.W.,[2] Huijbens R.J.F.,[1] Wenink M.H.,[1] Santegoets K.C.M.,[1] Vonk M.,[1] van den Berg W.,[1] Wagener F.A.D.T.G.,[2] Radstake T.R.D.J.[1]
1. Dept. of Rheumatology
2. Dept. of Pharmacology and Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Background: Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrosis of the skin and the internal organs. The etiology is still unknown but prominent features are vascular injury and chronic inflammation resulting in fibrosis. Accumulating evidence suggests a role for Toll-like receptor (TLR) mediated activation of dendritic cells

(DC). Heme Oxygenase-1 (HO-1) is a cytoprotective enzyme induced in response to stress factors like oxygen radicals or inflammation, both of which are abundantly present in SSc. We therefore investigated HO-1 protein expression and HO-activity and show that the altered IL-12 production in response to TLR ligands in SSc can be normalized via HO-1 induction.

Methods: 20 SSc patients were included. Patients were stratified as having diffuse SSc (dSSc) or limited SSc (lSSc) according to the extent of skin involvement. To assess HO-1 activity, bilirubin, a product of HO-1 activity, was measured in serum using the HPLC technique. After isolation from 50 ml of venous blood, monocytes were cultured to generate monocyte derived DCs (moDC). HO-1 levels were assessed with Western-Blot and qPCR before and after induction with the HO-1 inducer cobaltprotoporphyrin (CoPP). MoDCs were stimulated with several TLR ligands on day 6 with or without pre-incubation with CoPP for 24 hours. Levels of IL-10, TNFα, IL12p70 and IL-6 were measured in the supernatants using the Luminex Bead Array.

Results: Serum bilirubin levels and HO-1 Western-blot suggested a decreased HO-1 activity in SSc patients compared to healthy controls. Using the known HO-1 inducer, CoPP, the levels of the enzyme could be increased to the levels of healthy controls. A clearly increased production of IL-12p70 by moDCs from SSc patients was observed upon TLR4 and TLR8 stimulation. Interestingly via the induction of HO-1 the TLR responses were dampened, especially the IL-12 production in response to TLR4 and TLR8 ligands by moDCs from SSc patients.

Conclusion: The decreased HO-1 activity in SSc patients is intriguing due to all the natural inducers which are abundantly present. Here we show that induction of this enzyme could benefit SSc patients by ameliorating inflammation and therefore reducing the progression of the disease. Induction of HO-1 activity with a pharmacological inducer could thus offer a new therapeutic target in SSc.

P26 – Certolizumab Pegol in Combination With Methotrexate Or as Monotherapy Shows Cumulative Gains Over Time in Work and Home Productivity in Patients With Active Rheumatoid Arthritis

Westhovens R.,[1] Purcaru O.[2]

1. University of Leuven, Leuven
2. G3P-Outcomes and Access, UCB, Brussels, Belgium

Introduction: The negative impact of rheumatoid arthritis (RA) on productivity within and outside the home is a serious problem.

Aim: To evaluate the cumulative gains overtime in work and household productivity in patients with active RA following treatment with certolizumab pegol (CZP), a PEGylated anti-TNF.

Patients and Methods: Work and home productivity was assessed in three multi-center, double-blind, placebo-controlled clinical trials of CZP as combination therapy with methotrexate (MTX) (RAPID 1 and 2) (1,2) or as monotherapy (FAST4WARD) (3) in patients with active RA using the validated Work Productivity Survey (WPS-RA; administered every 4 wks starting at baseline [BL]). Annualized productivity scores for each treatment group were calculated by summing the productivity scores over the entire study duration starting Wk 4.

Results are presented as the difference in annualized scores between active and placebo groups. Missing data were imputed using the last observation carried forward (LOCF) method.

Results: 982, 619 and 220 patients were randomized into RAPID 1, RAPID 2 and FAST4WARD, respectively. Within each trial, treatment groups were comparable at BL in terms of household work and daily activities. The BL employment rate of subjects in each trial was 41.6%, 39.8%, and 39%, respectively. In all trials, improvements in productivity within and outside home were observed in the CZP arms as early as Wk 4, and maintained until study end (RAPID 1: 12 months, RAPID 2 and FAST4WARD: 6 months). Patients treated with CZP plus MTX or as monotherapy reported greater cumulative gains in full days of household and paid work, in more productive days at work and at home, and in daily activities compared with placebo (Table).

Conclusion: CZP reduces the impact of RA on both household and work productivity and improves the ability of RA patients to carry out family, social and leisure activities as early as Wk 4, through to the end of the study.

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Cumulative days gained over time		RAPID 1* (over 12 months)	RAPID 2* (over 6 months)	FAST4WARD** (over 6 months)
Work Productivity	Gain in full days	41.9	4.2	4.1
	Gain in more productive days	29.4	29.2	21
Home Productivity	Gain in full days	52.1	18.5	25.5
	Gain in more productive days	36.6	23.2	27.6
	Gain in social/family/leisure days	26.8	13.7	14.0

* RAPID 1 and RAPID 2: CZP 200 mg + MTX vs PBO + MTX
**FAST4WARD: CZP 400 mg vs PBO

P27 – Anti-mutated Citrullinated Vimentin Antibodies Seems to be Rather a Serological Marker of Psoriasis than of Rheumatoid Arthritis

Samoud S.,[1,2] Galai Y.,[2] Lachheb L.,[3] Bouajina I.,[4] Nouira R.,[3] Boukadida J.[1]

1. Laboratory of Immunology, Farhat Hached Hospital, Sousse
2. Laboratory of Immunology, Institut Pasteur de Tunis
3. Dept. of Dermatology
4. Dept. of Rheumatology, Farhat Hached Hospital, Sousse, Tunisia

Introduction: Antibodies directed against citrullinated vimentin are members of the family of autoantibodies reactive with citrullinated proteins and are among the most specific serological markers for the diagnosis of rheumatoid arthritis (RA). Vimentin modification is mediated by macrophages depending on pro-inflammatory signals. Psoriasis is both a chronic inflammatory skin and an autoimmune disease. Moreover, some longstanding psoriatic patients will develop psoriatic arthritis (PsA). This latter has some common characteristics with RA.

Aim: The aim of this study was to assess and compare the prevalence of anti-MCV in psoriasis patients and RA patients and sex and age matched healthy controls (HC).

Patients and Methods: Serum anti-MCV was measured in psoriasis (n=56) and RA (n=56) patients and 56 HC. Anti-MCV was detected by a second-generation ELISA. The activity of psoriasis was based on PASI score. Statistical analysis was performed using the non parametric *U- Mann-Whitney* test. Significance was assigned to *p* values lower than 0.05.

Results: Anti-MCV were detected in 52 of psoriatic patients (93%), 34 of patients suffering from RA (60.7%) but only in two HC. Their presence was significantly associated with psoriasis comparatively with healthy controls ($p<10^{-3}$) and RA patients ($p<10^{-3}$). Their presence was significantly associated (p=0.04) to the male sex (100% of men *versus* 85% of women). The presence of anti-MCV was also associated to the beginning of the disease at early age (p=0.037).

Conclusion: The high prevalence of anti-MCV in psoriasis could be explained by their lower specificity comparatively to the anti-cyclic citrullinated peptide antibodies in RA. In the other hand, they would reflect rather a generalized chronic inflammation than a joint inflammation. This result should push us to study the real role of macrophage, the producer of MCV, in psoriasis.

P28 – Chronic Arthritis Leads to Disturbances in the Bone Collagen Network

Caetano-Lopes J.,[1] Nery A.M.,[1,2] Canhão H.,[1,3] Duarte J.,[4,5] Cascão R.,[1] Rodrigues A.,[1,3] Perpétuo I.P.,[1] Abdulghani S.,[1] Amaral P.M.,[3] Sakaguchi S.,[6] Konttinen Y.T.,[7] Graça L.,[4,5] Vaz M.F.,[3] Fonseca J.E.[1,3]

1. Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Rheumatology Research Unit, Lisbon
2. Instituto Superior Técnico, Depto. de Engenharia de Materiais, Lisbon
3. Hospital de Santa Maria, Serviço de Reumatologia e Doenças Ósseas Metabólicas, Lisbon
4. Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Cellular Immunology Unit, Lisbon
5. Instituto Gulbenkian de Ciência, Oeiras, Portugal
6. Institute for Frontier Medical Sciences, Kyoto University, Dept. of Experimental Pathology, Kyoto, Japan
7. University of Helsinki, Dept. of Medicine, Helsinki, Finland

Objective: In this study we used a mice model of chronic arthritis to evaluate if bone fragility induced by chronic inflammation is associated with an imbalance in bone turnover and also a disorganization of the bone type I collagen network.
Methods: Serum, vertebrae and femur bones were collected from eight months old polyarthritis SKG mice and controls. Strength of the femoral bones was evaluated using three-point bending tests and density was assessed with a pycnometer. Bone turnover markers CTX-I and PINP were measured in serum. The organization and density of bone collagen was analyzed in vertebrae using second-harmonic generation (SHG) imaging with a two-photon microscope and trabecular bone microstructure was assessed by scanning electron microscope (SEM).
Results: Femoral bones of SKG mice revealed increased fragility expressed by deterioration of mechanical properties namely altered stiffness (p=0.007) and reduced strength (p=0.006), when compared to controls. In accordance intertrabecular distance and trabecular thickness as observed by SEM were reduced in SKG mice. PINP was significantly higher in arthritic mice (9.18±3.21ng/ml) when compared to controls (1.71±0.53ng/ml, p<0.001). Bone resorption marker CTX-I was 9.67±3.18ng/ml in arthritic SKG mice compared to 6.23±4.11ng/ml in controls (p=0.176). The forward-to-backward signal ratio measured by SHG was higher in SKG animals reflecting disorganized matrix and loose collagen structure compared to controls.
Conclusion: We have shown for the first time that chronic arthritis by itself impairs collagen metabolism and bone matrix architecture, probably due to disturbed bone remodeling. This effect might predispose to bone fragility fractures.

P29 – B-Cell Development In Sarcoidosis

Kamphuis L.S.J.,[1] van Zelm M.C.,[2] de Haas E.F.E.,[2] van Daele P.L.A.,[1,2] van Hagen P.M,[1,2] van Laar J.A.M.[1,2]
1. Dept. Internal Medicine, Section Clinical Immunology
2. Dept. of Immunology, Erasmus MC, University Medical Centre, Rotterdam, The Netherlands

Introduction: Sarcoidosis is a systemic disorder of unknown etiology characterized by formation of noncaseating granulomas. Patients with common variable immunodeficiency (CVID) can also develop noncaseating granulomas. This is frequently described as sarcoid-like syndrome. Those patients present with reduced numbers of memory B-cells.[1]

Malfunction of one of these steps in the B-cell differentiation may cause a defective humoral immune response.[2] The contribution of defects in B-cell differentiation in the pathophysiology of sarcoidosis is unknown. Considering the similarity of noncaseating granulomas in CVID and sarcoidosis, we presume a lack in the B-cell differentiation in sarcoidosis.
Aim: The immunophenotypical identification of peripheral B-cells from patients with sarcoidosis.
Patients and Methods: Peripheral blood from 15 therapy naive biopsy confirmed sarcoidosis patients and 10 controls were obtained. Immunophenotyping analysis on peripheral blood B-cell subsets was performed. Peripheral blood mononuclear cells were stained with antibodies against CD19, CD24, CD27, CD38, IgD and IgM and analyzed by flowcytometry. The Mann-Whitney test is used to show differences in the outcome variables.
Results: Peripheral levels of serum natural effector cells, IgM^+ memory B-cells and IgG^+ memory B-cells are significantly lower than those of controls (respectively P = 0.0043, P = 0.0087 and P = 0.0087), whilst plasma blasts and IgA^+ memory B-cells were increased (respectively P = 0.0253 and P = 0.0003).
Conclusion: Our study shows insufficiencies in B-cell differentiation in sarcoidosis patients. In physiological circumstances B-cells are activated directly by bacterial polysaccharide capsules independent of T-cell interference. These B-cells can induce rapid humoral responses characterized by the dominant production of IgM.[3] The decrease in natural effector cells and IgM^+ memory B-cells in our study suggest a dysregulation of the humoral response to bacterial pathogens.

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P30 – Interleukin-6 Promoter -174 G/C Polymorphism is Associated with Disease Severity and Cardiovascular Risk in Lupus Patients

Santos M.J.,[1,2] Fernandes D.,[1] Caetano-Lopes J.,[1] Perpetuo I.,[1] Vinagre F.,[2] Vidal B.,[1] Canhão H.,[1,3] Canas da Silva J.,[2] Fonseca J.E.[1,3]

1. Rheumatology Research Unit, Instituto de Medicina Molecular da Faculdade de Medicina de Lisboa, Lisboa
2. Rheumatology Dept., Hospital Garcia de Orta, Almada
3. Rheumatology and Bone Metabolic Diseases, Hospital Santa Maria, Lisboa, Portugal

Background: Systemic Lupus Erythematosus (SLE) is an autoimmune condition of unknown etiology with both genetic and environmental features being implicated in the disease development. SLE patients exhibit alterations in cytokine production that may be important in disease pathogenesis. There is evidence that interleukin-6 (IL-6) gene polymorphisms are relevant in the modulation of IL6 expression, an essential regulator of the acute phase response. Consequently, these polymorphisms may be associated with the susceptibility for SLE development or with distinct disease features.

Aims: To investigate the association of -174 G/C polymorphism in the promoter region of the IL-6 gene with SLE susceptibility and with its clinical manifestations in Portuguese patients.

Patients and Methods: Consecutive patients who fulfilled criteria for the diagnosis of SLE were evaluated and compared with healthy controls from the same ethnic background. IL-6 promoter -174 G/C polymorphism was determined by Restriction Fragment Length Polymorphisms.

Results: One hundred and twenty SLE patients (96% females; 86% Caucasians; mean age 42.3±14y) and 103 healthy controls (92% females; 99% Caucasians; mean age 43.2±14.5y) were studied. The -174 IL-6 genotype frequencies were similar in Portuguese SLE patients and controls (10.3% CC, 51.6% GC, 37.1% GG in patients and 10% CC, 44.2% GC, 45.8% GG in controls). The occurrence of proteinuria (58.3% vs 25.9%; p=0.04), irreversible damage (50% vs 24%; p=0.02) and metabolic syndrome (62.5% vs 20%; p=0.01) was higher among patients with the -174CC genotype, as compared to those with the -174GG genotype.

Additionally, patients with the -174CC genotype had significantly higher erythrocyte sedimentation rate (ESR), higher total and LDL cholesterol levels and higher serum uric acid.

	-174 CC	-174 GC	-174 GG	p value
ESR mm/h	44±32	28±20	30±25	<0.05
Total cholesterol mg/dl	228±64	195±42	187±36	0.01
LDL cholestrol mg/dl	139±57	117±34	109±29	0.03
Uric acid mg/dl	6.7±2.8	4.1±1.1	4.7±1.4	0.004

Conclusion: IL-6 promoter –174G/C polymorphism is not a major susceptibility factor for SLE, but predisposes to distinct clinical features. The CC genotype seems to be associated with more severe SLE and with several cardiovascular risk factors, including metabolic syndrome. The association of IL-6 -174G/C polymorphism with these features is likely to be mediated through inflammatory mechanisms, including genetically determined IL-6 response.

P31 – Imatinib Successful in Progressive Systemic Sclerosis Associated with Metastatic Gastro-Intestinal Stroma Cell Tumor

Bonte-Mineur F.,[1] van Daele P.L.A.,[2] van Hagen P.M.,[2] Koeyers W.J.,[3] Thio H.B.,[3] van Paassen H.C.,[1] van Laar J.A.M.[2]

1. Dept. of Rheumatology, Sint Franciscus Gasthuis, Rotterdam
2. Dept. of Internal Medicine and Immunology
3. Dept. of Dermatology, Erasmus University Medical Centre, Rotterdam, The Netherlands

Introduction: Several studies have demonstrated an increased frequency of cancer in patients with systemic sclerosis (SSc), especially lung and breast cancers. The pathogenesis of the association between SSc and cancer is not fully established. Treatment of the primary tumor gives variable responses on the evolution of SSc[1-4]. Gastro-intestinal stroma tumor (GIST) rarely presents with paraneoplastic symptoms and an association with SSc has never been reported. Imatinib, a novel tyrosine kinase inhibitor and first line treatment for advanced GIST, recently is reported effective in a patient with therapy-refractory SSc[5,6]. We describe a patient with a combination of progressive SSc and a GIST. We hypothesized that SSc in this patient might also respond to imatinib.

Aim: To evaluate the effect of treatment with imatinib on both tumor load and SSc related symptoms.

Patient and Methods: A 51-year old woman was diagnosed with severe Raynaud's phenomenon and SSc, one year prior to diagnosis of a 22 cm CD 117, CD34 and C-KIT mutation positive GIST. Chirurgical resection of the tumor led to stabilization of the SSc. Two years later however she developed progressive debilitating SSc with a modified Rodnan skin thickness score (MRSS) rapidly deteriorating to 39 impeding the function of hands and arms, and pulmonary fibrosis. Suspecting recurrence of her GIST, further workup was done. Histology from a solitary liver lesion revealed a relapse of the GIST, without other intra-abdominal lesions. Daily treatment with 400mg imatinib was initiated and therapeutic efficacy was monitored by CT-scans and MRSS.

Results: Three weeks after treatment started, impressive subjective improvement occurred.

The patient noticed improved function of hands and arms, along with less facial stiffness. Her MRSS decreased to 26.

Conclusion: This case shows that SSc might be associated to GIST. We believe that Imatinib treatment reduced SSc symptoms by blocking TGF-β and PDGF-signalling of the fibroblasts[5-8], although, if the SSc in this patient is regarded as a paraneoplastic disease, an indirect effect due to tumor reduction cannot be excluded. To our knowledge, this is the first reported case of SSc associated with a GIST improving on treatment with imatinib.

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P32 – FOXP3+ Regulatory T Cells are Decreased in Peripheral Blood of JDM Patients During Disease Remission

Elst E.,[1] Prakken B.,[1] van Royen-Kerkhof A.,[2] van Schieveen C.,[1] Vercoulen Y.[1]

1. Laboratory of Paediatric Immunology and Rheumatology
2. Dept. of Clinical Paediatric Immunology and Rheumatology, Wilhelmina Children's Hospital of the University Medical Center, Utrecht, The Netherlands

Introduction: Autoimmune disease can be the result of improper immune suppression. Thymus-derived naturally occurring CD4+CD25+ regulatory T-cells (Tregs) do not proliferate upon antigen-specific stimulation, but instead inhibit activation of effector T cells, thereby regulating inflammatory responses. Development and function of CD4+ Tregs is regulated by transcription factor forkhead box P3 (FOXP3). Glucocorticoid-inducible tumor necrosis family-related receptor (GITR) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) are expressed on Tregs and related to Treg function. Juvenile dermatomyositis (JDM) is a clinical well-defined auto-immune disease in which the immune system targets the microvasculature of the skeletal muscle and skin, leading to myopathy and typical skin rash.

In this study we aimed to determine whether the amount and functional capacity of Tregs plays a role in immune dysregulation in JDM patients.

Patients and Methods: Eleven JDM patients (6 remission, 5 active disease) and six HC (< 18 years) were enrolled. Expression of CTLA-4 and GITR in CD4+ cells, CD4+ FoxP3+ cells, CD4+CD25bright and CD4+FOXP3 T cells was determined using flowcytometry. Functional suppressive capacity of CD4+FOXP3 + Tregs was determined in a co-culture of anti-CD3 stimulated PBMC and CD4+

CD25+CD127low T cells at different ratios.
Results: FOXP3 expression by CD4+ T cells was comparable between JDM patients and HC. However, during disease remission FOXP3 expression was significantly decreased compared to active disease, as well as compared to HC. Expression of CTLA-4 on FOXP3+ T cells in remission was similar to active disease and HC. Both FOXP3+ and FOXP3- CD4+ T cells express higher levels of GITR during active disease. Suppressive capacity was tested in 2 patients in remission. We found up to 55% suppression of proliferation by CD4+CD25+CD127- T cells in a ratio of 1:1.
Conclusion: Although Tregs from JDM patients display normal suppressive function in vitro, patients in the remitting phase of disease have lower levels of CD4+FOXP3+ Tregs in peripheral blood compared to active disease and HC. This suggests that JDM patients could be more susceptible to either development, or relapse of disease, due to low Treg numbers. Further comparison of Treg suppressive capacity between patients in remission and active disease, compared to age-related HC is required to support these findings.

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P33 – Case: Cytokine Profile in the Colon of a Patient With Intestinal Behçet's Disease

Kappen J.H.,[1] Dik W.A.,[2] Dingjan G.M.,[2]
van Daele P.L.A.,[1,2] Hooijkaas H.,[2]
van Hagen P.M.,[1,2] van Laar J.A.M.[1,2]
1. Dept. of Internal Medicine, Section Clinical Immunology
2. Dept. of Immunology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Introduction: The immunopathology of Behçet's disease (BD) remains unraveled. BD is a systemic vasculitis with oral and genital aphthous ulcers, skin lesions and ocular inflammation. Since the successful introduction of TNF-blockers it was hypothesized that Th1-cytokines are key players in BD.[1,2] Data on cytokine levels especially in affected tissues is limited.[3] We therefore present data on cytokine expression in the colon of a patient with BD colitis.[4]
Case: In a 37-year old BD patient with severe colitis, traditional immunosuppressive treatment failed. Initially, TNF-blockage successfully reduced the severity of the colitis, but therapy refractory relapses occurred. There were no antibodies against infliximab or adalimumab detected. Eventually, high dose infliximab (10mg/kg) and intravenous immunoglobulins (IVIG's) led to disease regression in order to perform a hemi-colectomy.

The patient's condition improved significantly and IVIG's were tapered whilst continuing TNF-blockage.
Methods: Cytokines were evaluated in the resected colon by analyzing mRNA expression levels of cytokines.[5] Healthy and diseased tissue could macro- and microscopically be separated. Because of the extensive prior treatment the patient served as

internal control.

Results: In the figure the mRNA expression of cytokines in diseased colon relative to those of healthy colon is presented. This can be interpreted as a reflection of local cytokine levels.

A Th1 and Th17-skewed pattern (elevated IFN-γ and TNF-α, and IL-17A, respectively) is seen.

Conclusions: In colonic tissue of intestinal BD, IFN-gamma, TNF-alpha and IL-17A appear key cytokines, even in a treated patient.

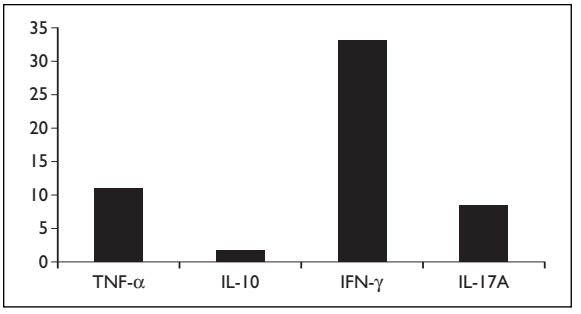

Figure 1. Cytokine mRNA ratio (affected/ healthy tissue) in a patient with intestinal Behçet's disease.

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P34 – TH1, TH2 AND TH17- DERIVED CYTOKINES LEVELS IN SYNOVIAL FLUID OF RA: RELATION WITH SYNOVIAL LYMPHOID NEOGENESIS AND EROSIVE DISEASE

Canete J. D.,[1] Celis R.,[1] Descalzo M.A.,[2] Pablos J.L.,[3] Sanmarti R.[1]

1. Rheumatology Dept, Hospital Clinic, Barcelona
2. Unidad de Investigación, Fundación Española de Reumatología
3. Rheumatology Dpt, Hospital Clinic, Barcelona, Spain

Background: Synovial lymphoid neogenesis (LN) in rheumatoid arthritis (RA) has been associated with refractory disease but not with the presence of autoantibodies. B cells in LN could also drive antibody-independent Synovial inflammation through the development of T cells responses or enhances cytokine production.

Purpose: To analyze 1) Th1, Th2 and Th17 cytokine levels in Synovial fluid of RA patients regarding the presence or not of Synovial tissue lymphoneogenesis; 2) To determine whether baseline levels of cytokines are associated to erosive disease.

Method: Arthroscopic Synovial biopsy specimens from patients with RA were classified as LN+ or LN- by immunohistochemistry for CD3, CD20, T/B cell segregation and peripheral node addressin (PNAd)-positive HEV in relation to the size of lymphoid aggregates. Synovial fluid obtained at the time of the arthroscopy was analyzed by duplicate using the Procarta Cytokine Assay Kit (Panomics, Inc, Freemont, CA) for IFN-gamma, IL-4, IL-7, IL-10, IL12p40, IL-17, IL-23, IL-1beta, TNF-alpha and IL-6. Relevant clinical and biological data of patients were recorded at inclusion and at the end of follow-up. Patients were further classified as having erosive or non erosive RA, as defined by having more than 2 radiographic erosive lesions in at least 2 different joints.

Results: 65 RA patients (68% female, 71% RF+, 51% ACPA+) with matched Synovial tissue and Synovial fluid samples were included with a follow-up, median (p25-p75) of 107 months (83-176). 78% of them had erosive at end of follow-up, 33 (51%) RA patients were LN+ and 32 (49%) LN-, without significant differences in disease duration, RF, ACPA, CRP and DAS28. Also, no significant differences were found in the levels of all cytokines between LN+ and LN- patients. Chi-square test showed a association between IL-1beta baseline levels and erosive RA (p=0.047). After Multivariable logistic regression adjusted for DAS28 at baseline, DAS28 increase, disease duration, CRP at baseline and anti-TNFa therapy, all of them with a significant association ($p<0.05$) with erosive disease, only IL-23 (p=0.02) and TNFalpha (p= 0.001) levels were associated with erosive RA.

Conclusion: There are no significant differences

Table 1. Frequency of S alleles in patients with and without anti-CFFCP1 and CCP2 antibodies

	Absence of S2 Alleles	Presence of S2 Alleles	OR (CI 95%)	Absence of S3D Alleles	Presence of S3D Alleles	OR (CI 95%)	Absence of S3P Alleles	Presence of S3D Alleles	OR (CI 95%)
CFFCP1 (+)	73%	96%	9,3 (1,2-72)*	82 %	53%	0,24 (0,08-0,75)*	79%	78%	0,93 (0,37-2,33)
CCP2 (+)	67 %	78%	1,7 (0,63-4,7)	74%	40%	0,24 (0,08-0,73)*	59%	76%	2,15 (0,97-4,8)¶

* p=0,001; ¶ p=0,058

in the T-cell derived and proinflammatory cytokine levels in synovial fluid of RA with and without Synovial lymphoid neogenesis. This finding suggests that LN does not modify the pattern of expression of theses cytokines, although analysis of cytokine expression in synovial tissue remains to be explored. On the other hand, Synovial fluid levels of TNFalpha and IL-23, but not IL-17 levels are associated with erosive disease, highlighting the relevance of theses cyokines in the pathogenesis of RA.

P35 – S2 Shared Epitope Alleles (QKRAA and/or DKRAA) Are Strongly Associated with Antibodies Against Chimeric Fibrin/Fillagrin Citrullinated Peptides in Early Rheumatoid Arthritis

Cañete J.D.,[1] Ercilla G.,[2] Gomez-Centeno T.,[3] Gomez-Puerta J.A.,[1] Gomara M.J.,[4] Graell E.,[3] Haro I.,[4] Sanmarti R.,[1] Vazquez I.,[3] Viñas O.[2]

1. Arthritis Unit, Rheumatology Dept.
2. Immunology Dept., Hospital Clinic, Barcelona
3. Rheumatology Dept., Hospital Parc Tauli, Sabadell
4. Unit of Synthesis and Biomedical Applications of Peptides, IQAC-CSIC, Barcelona, Spain

Background: In recent years a new classification of HLA-DRB1 alleles based on the amino acid sequence at position 70-74 has been proposed in order to explain in a better way the shared epitope (SE) hypothesis (*du Montcel ST, Arthritis Rheum 2005; 52: 1063-8*). Alleles containing the SE are clearly associated with RA positive for anti-citrullinated peptide antibodies (ACPA). We previously demonstrated a high sensitivity and specificity of a non-commercial ELISA test containing synthetic chimeric fibrin/filaggrin citrullinated peptides (CFFCP) *(Perez ML J Med Chem 2007; 50: 3573-84)*. One of these CFFCP antibodies (CFFCP1) are associated with greater radiographic progression in early RA (*ACR 2008*[1]).

Objective: To analyze the association of antibodies against CFFCP1 with SE alleles according to the new classification in patients with early RA and to compare its association with anti CCP2 commercial test.

Methods: One hundred and eighteen patients with early RA (< 2 years of disease duration) were included. Serum anti CFFCP1 and anti CCP2 (Immunoscan, Eurodiagnostica) antibodies were measured at baseline, previous the introduction of DMARDs in all patients.

HLA-DRB1 typing was performed by direct DNA sequencing. HLADRB1 alleles were divided in two groups according to the presence of RAA sequence al position 72-74 defining S and X alleles. S alleles were subdivided into four categories according to the aminoacid sequence at position 70 or 71: *S1* (QARAA, DERAA), *S2* (QKRAA, DKRAA), *S3D* (DRRAA) and *S3P* (QRRAA, RRRAA).

Results: 118 (82 % Female) patients were included, with a mean age of 53.8 ± 15 years and a mean disease duration of 9.2 ± 5.8 months. DAS28 at baseline was 5.7 ± 0.9.

Rheumatoid factor was positive in 73.7% of patients, CCP2 was positive in 69% and anti CFFCP1 in 72 % of patients.

In Table 1 we have shown the frequency (percentage) of the different S alleles in patients with and without these antibodies. There was no association among the presence of S1 alleles with CCP2 or CFFCP1 antibodies.

S2 alleles, that contains QKRAA (DRB1*0401) were strongly associated with anti-CFFCP1 but not with anti CCP2 status. S3D alleles considered as protective alleles were negatively associated with both CFFCP1 and CCP2 status. A non-significant

trend between the association with S3P (that confers an intermediate risk alleles) and CCP was found, but not with CFFCP1 status.
Conclusions: In a Spanish population of early RA, CFFCP1 and CCP2 status differs in the association with different SE alleles. CFFCP1 status is strongly associated with S2 alleles, which has been associated with a more aggressive disease in patients with RA. CFFCP1 status could be a better marker of poor prognosis in early RA than CCP2 status.

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P36 – The LTA 252 A/G Polymorphism Might be a Marker for Rheumatoid Arthritis Susceptibility

Fernandes D.,[1] Caetano-Lopes J.,[1] Perpétuo I.,[1] Vidal B.,[1] Canhão H.,[1,2] Santos M.J.,[1,3] Fonseca J.E.[1,2]
1. Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa
2. Rheumatology and Bone Metabolic Diseases Dept., Hospital Santa Maria, Lisboa
3. Rheumatology Dept., Hospital Garcia de Orta, Almada, Portugal

Introduction: Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are chronic inflammatory diseases that affect predominantly women. Recently, cardiovascular disease (CVD) has emerged as a significant contributor to morbidity and mortality in patients with RA and SLE.
Several polymorphisms have been associated with CVD, Among these are the -308 position of the promoter region of the Tumor Necrosis Factor (TNF) gene, the position +252 of the Lymphotoxin α (LTA) gene and at the -174 position of the promoter of the interleukin (IL)-6 gene. The first two are associated with an increased level of the inflammatory marker C-reactive protein.
The aim of this work was to investigate the occurrence of TNF -308 G/A, LTA 252 A/G and IL6 -174 C/G polymorphisms in RA and SLE Portuguese patients.
Patients and Methods: Patients who fulfilled criteria for the diagnosis of RA and SLE were evaluated and compared with healthy controls, being all Caucasians females. Blood have been collected into EDTA-containing tubes and the polymorphisms were determined by Restriction Fragment Length Polymorphisms (RFLP).
Results: Sixty women with RA, ninety eight women with SLE and eighty eight healthy controls were studied.

The frequency of the LTA 252 AA genotypes were higher in RA as compared to controls (63.6% vs 28.4%; $p<0.05$), but not in SLE patients when compared to controls (37.5% vs 28.4%, $p>0.05$). There were no significant differences in the frequency of the studied polymorphisms between RA, SLE patients and healthy controls regarding TNF -308 G/A and IL6 -174 C/G polymorphisms.
Conclusion: The LTA 252 A/G polymorphisms might be a marker for RA susceptibility. TNF -308 G/A and IL6 -174 C/G polymorphisms do not seem to be a susceptibility factor for RA or SLE Portuguese female patients. These results should be confirmed with a larger sample.

P37 – Tuberculosis Safety Monitoring of the Use of Monoclonal Antibodies Against TNF in Portuguese Rheumatoid Arthritis Patients Based on an Electronic Medical Chart and Registry (BioReportAR): Experience of a Single Centre

Polido-Pereira J.M.,[1] Rodrigues A.M.,[1] Ferreira A.I.,[1] Carvalho A.M.,[1] Saavedra M.J.,[2] Barros R.,[2] Catita C.,[2] Marques A.,[2] Canhão H.,[1] Viana de Queiroz M.,[2] Fonseca J.E.[1]
1. Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa, Universidade de Lisboa and Santa Maria Hospital, Lisboa
2. Serviço de Reumatologia e Doenças Ósseas Metabólicas, Hospital de Santa Maria, Lisboa, Portugal

Introduction: Monoclonal antibodies against TNF have been associated to the occurrence of severe infections, particularly reactivation of latent tuberculosis (TB). Due to the high prevalence of TB in Portugal, the Portuguese Society of Rheumatology developed guidelines for the screening and treatment of latent TB in patients treated with biologics. Electronic medical charts that automatically download data into a registry can provide a structured approach to the medical practice and improve the surveillance of adverse effects.
Aim: To present the results of TB screening and sa-

fety of RA patients treated with anti-TNF monoclonal antibodies using an electronic database registry developed by the Portuguese Rheumatology Society- BioReportAR.

Patients and Methods: Data was captured directly from the BioReportAR, including RA patients treated with infliximab or adalimumab in Hospital de Santa Maria, Lisboa.

Results: 56 patients, 85,7% females, mean age 53.2±14.1 years. Treated with infliximab or adalimumab with a mean duration of treatment 1198.6 days, corresponding to 183.8 patient years. Regarding latent TB screening, 75% patients had a PPD test below 5 mm (40.5% repeated the test and the result was also below 5mm), 3.6% between 5 and 10 mm and 14.3% higher than 10 mm. The chest x-ray was suggestive of TB in 1.79% patients and suspicious in 3.6%; 8.9 % had nonspecific pulmonary infiltrates and 76.8% had a normal exam. 26.8% of the patients were submitted to TB chemoprophylaxis. 35% of TB chemoprophylaxis was prescribed in PPD negative patients and in these cases contact history was the main determinant for therapy decision. No severe adverse effects related to the use of isoniazide were reported. There were no reports of TB reactivation, but there was a report of Mycobacterium avium infection in a patient with long-standing RA treated with infliximab.

Discussion: BioReportAR in our centre showed that 26.8 % of patients were treated with TB chemoprophylaxis before starting TNF monoclonal antibody, a third of them in PPD negative patients. In negative PPD patients, PPD retesting was not useful. Neither isoniazide severe adverse effects nor TB reactivation cases were reported.

BioReportAR is a useful tool for the quality assessment of medical practice and provides an accurate assessment of safety data.

P38 – Flow Cytometric Characterisation of Freshly Isolated and Culture Expanded Human Synovial Cell Populations in Patients With Chronic Arthritis

Van Landuyt K.B.,[1] Jones E.A.,[2] McGonagle D.,[2] Luyten F.P.,[1] Lories R.J.[1]

1. Laboratory for Skeletal Development and Joint Disorders, Dept. of Musculoskeletal Sciences, Division of Rheumatology, Katholieke Universiteit Leuven, Leuven, Belgium

2. Academic Unit of Musculoskeletal Disease, Leeds Institute of Molecular Medicine, University of Leeds, UK

Introduction: The synovium is a major target tissue in chronic arthritis and is intensively studied at the cellular and molecular level.

Aim: We aimed to develop flow cytometry for the quantitative analysis of synovial cell populations pre and post culture and to characterize mesenchymal cell populations residing in the inflammatory synovium.

Patients and Methods: Knee synovium biopsies from 31 patients with rheumatoid arthritis or spondyloarthritis, and from 15 controls were treated in a short, standardized tissue digestion procedure. Stored thawed digests were analyzed with flow cytometry including live-dead staining and use of the markers CD45, CD3, CD14, CD20, CD34, CD73, CD105, CD90, CD146, CD163 and HLA-DR. The influence of the digestion method on the detection of the different surface markers was studied separately. Cell expansion cultures were set up and a MSC-related surface marker profile in passages 3 and 6 was obtained.

Immunohistochemistry for CD34 and vWF was done to obtain additional data on synovium vascularity.

Results: The cell yield and viability normalized to tissue weight were significantly higher in inflammatory arthritis than in controls. Within the hematopoietic CD45-positive populations, we found no differences in relative amounts of macrophages, T-lymphocytes and B-lymphocytes between patient groups. Within the CD45-negative cells, more CD34-positive cells were seen in controls than in arthritis. Culture expanded cells were found to fulfill most of the multipotent mesenchymal stromal cell marker profile, except for CD34 negativity.

Detection of peripheral blood macrophage and B-cell markers was decreased after enzymatic exposure and mechanical forces, respectively, but stromal markers were not affected.

Conclusions: Flow cytometry can distinguish synovial cell populations in tissue digests. The preparation method can influence the detection levels of macrophage and B-cell populations.

However, stromal cell markers seem not affected and quantification is possible, supporting flow cytometry tissue analysis as a tool to study these cell populations in arthritis.

P39 – Patients with Severe Acute Pancreatitis Treated at Intensive Care Unit have Highly Aberrant Monocyte Signaling Profile Assessed by Phospho-Specific Flow Cytometry

Oiva J., Mustonen H., Kylänpää L., Kyhälä L., Siitonen S., Puolakkainen P., Kemppainen E., Repo H.
Helsinki University Central Hospital, Depts. of Surgery, Clinical Chemistry, and Medicine, Helsinki, Finland

Aim: To outline signaling profiles and transmigration capacity of monocytes of patients with severe acute pancreatitis.
Patients and Methods: Thirteen patients with severe acute pancreatitis (AP).

Phosphorylation of NF-κB and p38, Stats1,3,5,6, and ERK1/2 in were studied using phospho-specific whole blood flow cytometry.[1,2] Transmigration of monocytes was studied using Transwell cell culture inserts covered with EA-HY cells.
Results: Phosphorylation levels of NFκB induced by TNF, bacterial lipopolysaccharide, muramyl dipepetide, *E.coli*, *S.aureus,* and *S.epidermidis* were lower in patients' monocytes than monocytes of healthy subjects, whereas MAP kinase p38 phosphorylation levels were normal. Phosphorylation levels induced by interleukin(IL)-6 in Stat1 and Stat3, and those by combination of phorbol 12-myristate 13-acetate and calcium ionophore A23187 in ERK 1/2, members of a MAP kinase family, were depressed in patients' monocytes, whereas those induced by GM-CSF in Stat5 and by IL-4 in Stat6 were normal. Transmigration% of patients' monocytes was lower than that of reference monocytes.
Conclusions: Monocytes of patients with severe AP show impaired NFκB activation, which may increase susceptibility to infections, normal p38 activation and depressed Stat3 activation, which may contribute to maintenance of inflammation, and impaired ERK1/2 activation, which may depress monocytes' transmigration and increases risk of infection.

Monitoring of monocyte signaling profiles may aid to find new therapeutic approaches and predictors of outcome of severe AP.

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P40 – Gene Array Profiling in Dermatitis Herpetiformis Skin Lesions

Cozzani E.,[1] Parodi A.,[1] Dolcino M.,[2] Bason C.,[3] Lunardi C.,[3] Puccetti A.[2]
1. Dermatology Dept., University of Genova
2. Institute G. Gaslini, Genova
3. Dept. of Internal Medicine, University of Verona, Italy

Introduction: Dermatitis herpetiformis (DH) is a rare disease considered a cutaneous manifestation of gluten-sensitive enteropathy (CD). DH is an IgA mediated blistering skin disease characterized by the presence of granular deposits of IgA in papillary dermis. The disease is considered to be gluten dependent and a gluten-free diet (GFD) is therefore the treatment of choice. The question why some patients with CD develop DH is still unanswered.
Aim: To clarify this point we compared the gene expression profiles in skin biopsies from CD patients with and without DH.
Patients and Methods: A gene array analysis was performed using skin biopsies from 6 patients with DH and 6 patients without clinical and histologically documented skin involvement. All the patients were on free diet and DH patients were not on Dapson treatment. Gene array probing was performed with the Human Genome U133A GeneChip representing 14,500 well-characterized human genes and including more than 22,000 probe sets. The different gene expression patterns were analyzed using the Gene Spring software, version 10.0.
Results: We found that 555 transcripts were differently modulated in DH samples compared to CD samples. Particularly 252 and 303 transcripts resulted respectively up- and down-regulated. Modulated genes were classified in several clusters on the basis of their biological function, including immune response, apoptosis, cell growth, proliferation and differentiation, inflammatory response, production and remodelling of the extracellular matrix. IL-8 resulted up-regulated in DH samples. It is a strong chemoattractant for neutrophils and a potent inducer of PLAU in cultured human skin. Interestingly PLAU was up-regulated in DH samples. Several proapoptotic genes were also up-regulated such as: DIDO1, PDCD10, and GSPT1. In

DH samples we observed also an overexpression of several extracellular matrix peptidases including MMP9, ADAM9 and ADAM19. Among the extracellular matrix components fibrillin, that may be one of the structural component to which IgA reactive deposits bind in DH skin, resulted overexpressed.
Conclusion: The gene array analysis shows a profound difference at transcriptosome level between the skin of celiac patients with and without DH. This may be helpful in understanding the pathogenesis of DH and in designing new therapeutic strategies.

P41 – STAT3 IS CONSTITUTIVELY ACTIVATED IN MONOCYTES AND LYMPHOCYTES OF PATIENTS WITH EARLY RHEUMATOID ARTHRITIS

Aittomäki S., Alanärä T., Siitonen S., Repo H., Leirisalo-Repo M.
Depts. of Clinical Chemistry and Rheumatology, Helsinki University Central Hospital, Helsinki, Finland

Aim: To outline signaling profiles of leukocytes in early rheumatoid arthritis (RA) and spondyloarthropathy (Spa).
Patients and Methods: Ten patients with untreated early RA and eight patients with active Spa were included. Reference samples were obtained from healthy subjects (n=10). Phosphorylation of signaling proteins was studied using phospho-specific whole blood flow cytometry (1,2). In unstimulated samples, marker was set so that proportion of positive cells was < 5% in healthy controls.
Results: In unstimulated RA samples, proportion of Stat3-positive monocytes was > 5% in 7/10 patients and in Spa samples in 1/8 patients (respective range of proportions <5.0-42.6%vs<5-18.1%,p=0.043, Mann-Whitney-U-test). Respective results were among other cell types: all lymphocytes 9/10 RA patients vs 3/8 Spa patients (range <5.0-40.0%vs<5.0-11.6%,p=0.003); CD4-T-cells 5/8 RA patients vs 1/8 Spa patients (<5.0-78.6%vs<5.0-8.1%,p=0.003); CD8-T-cells 2/8 RA patients vs 1/8 Spa patients (<5.0-17.3%vs<5.0-8.5%,p>0.05); CD19-B-cells 4/8 RA patients vs 2/8 Spa patients (<5.0-17.6%vs<5.0-11.7%,p>0.05). In stimulated samples, phosphorylation levels of monocyte NFkB and p38, monocyte ERK1/2, Stat5 and Stat6, lymphocyte NF-κB and Stat3, CD4-T-cell Stat3, CD8-T-cell Stat3, and neutrophil p38 were comparable in patients and healthy subjects.
Conclusions: In patients with untreated early RA, Stat3 is frequently activated in monocytes and CD4-T-cells, and occasionally in CD8-T- and CD19-B-cells. The possibility that Stat3-profile correlates with anti-T-cell, -B-cell and –TNF therapies warrants prospective studies.

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P42 – SAFE USE OF ETANERCEPT IN AN ANKYLOSING SPONDYLITIS PATIENT WITH A KIDNEY TRANSPLANT TREATED WITH TACROLIMUS AND MICOPHENOLATE MOPHETIL

Rodrigues A.,[1,2] Resende C.,[1,2] Polido-Pereira J.,[1,2] Sousa E.,[1,2] Guerra J.,[3] Viana Queiroz M.,[2] Canhão H.,[1,2] Fonseca J.E.[1,2]
1. Rheumatology Research Unit, Instituto Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisboa
2. Rheumatology and Bone Metabolic Diseases Dept., Hospital de Santa Maria, Lisboa
3. Nephrology and Renal Transplant Dept., Hospital de Santa Maria, Lisboa, Portugal

Background: Etanercept in association with other immunosuppressive therapies has been increasingly used in acute graft-versus-host disease, with no significant additional adverse effects. However, there is no data regarding its use in renal transplanted patients with active rheumatic diseases. We report the safe use of etanercept in an ankylosing spondylitis (AS) patient with a renal transplant treated with tacrolimus and micophenolate mophetil.
Case Report: Male, 46 years old with AS that started at the age of 9 as an HLA-B27 positive enthesitis-related arthritis, with axial and peripheral involvement, treated for several years with non-steroidal anti-inflammatory drugs (NSAID). At 35 years old, due to persistent severe disease he required bilateral knee and hip replacement. In 2004, the pa-

tient developed end-stage renal failure secondary to the use of NSAIDs, leading to hemodyalisis.

He also had a flare of his rheumatic disease with arthritis of both wrists and ankles, BASDAI of 7.44, BASFI of 8, visual analogical scale (VAS) for nocturnal vertebral pain of 40mm, erythrocyte sediment rate (ESR) of 30mm and C-reactive protein (CRP) of 2,3mg/dl. It was decided to start etanercept with improvement of arthritis, BASDAI (3,34), BASFI (6,2), VAS for nocturnal vertebral pain of 1mm, ESR (5mm) and CRP (0,5mg/dl), three months later.

Other than an episode of cellulitis, no other side effects were reported. One year after starting etanercept, the patient was submitted to renal transplant and this drug was interrupted. During the following 4 years, he had persistent high disease activity. In January 2009, it was decided to restart etanercept along with the other immunosupressives (tacrolimus and micophenolate mophetil) for the prevention of organ rejection. Again, at three months, a reduction of the number of tender and swollen joints (4 to 0), BASDAI (6,78 to 2,8), BASFI (8,42 to 7,9), nocturnal vertebral pain VAS (70 to 10 mm), ESR (25 to 17 mm) and CRP (3 to 0,39mg/dl) was observed. At 6 months of treatment the patient maintains a good clinical response with no infections or toxicities reported.

Conclusion: Rheumatic patients undergoing intensive immunosuppressive treatment to avoid renal transplant rejection can use concomitantly etanercept, under rigorous clinical surveillance.

P43 – Cytokine Profile in Rheumatoid Arthritis Patients Under Different Therapies

Perpétuo I.,[1] Fernandes D.,[1] Caetano-Lopes J.,[1] Rodrigues A.,[1,2] Polido-Pereira J.,[1,2] Viana Queiroz M.,[2] Fonseca J.E.,[1,2] Canhão H.[1,2]
1. Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa, Lisbon
2. Rheumatology and Bone Metabolic Diseases Dept., Hospital de Santa Maria, Lisbon, Portugal

Introduction: Both anti-TNF and anti-IL-6 receptor therapies reduce disease activity by inhibiting TNF and IL-6 signalling, but little is known about the effects of these drugs on the production of other cytokines. Moreover, it is known that inflammatory environment potentiates bone resorption, modulating the balance between receptor activator for nuclear factor κ B ligand (RANKL) and osteoprotegerin (OPG). Again, data about the effects of these therapies on bone metabolism are insufficient.

Aim: The aim of this work was to assess the levels of several key players of inflammation and bone metabolism in RA patients treated with different drugs.

Patients and Methods: A total of 42 female patients with RA were recruited, 7 of them were on corticosteroid therapy without disease-modifying antirheumatic drugs (DMARDs), 12 on methotrexate (MTX), 13 on anti-TNF and 10 on tocilizumab. Proteins in serum were assayed by Enzyme Linked Immuno-Sorbent Assay (ELISA) or by a multiplex bead-based immunoassay.

Results: Both anti-TNF and tocilizumab therapy significantly decreased the circulating levels of IL-10 and of the neutrophil chemottractant IL-8 when compared to the other groups. IL-6, the acute phase response cytokine, was undetectable in the majority of the patients except on those under tocilizumab therapy (21,9pg/mL). Biological treated patients have significantly reduced levels of OPG, RANKL and RANKL/OPG ratio. Adiponectin levels were dependent on body mass index (BMI) regardless of the ongoing therapy or of the disease activity. The disease activity score (DAS28) was 3.26 in biological-treated patients, 4.85 in MTX treated patients and 5.31 in corticosteroid treated patients.

Conclusion: Differences in the cytokine profile between therapies were found however, they might be more dependent on disease activity rather than on differences in the treatment regimes analysed.

P44 – Microscopic Polyangiitis (MPA) in a Patient With Rheumatoid Arthritis

Videira T., Terroso G., Brandão F., Simões-Ventura F.
São João's Hospital Rheumatology Service and Oporto Faculty, Portugal

Introduction: Rheumatoid arthritis (RA) is a systemic auto-immune disorder that primary involves joints and although renal disease can occur, rapidly progressive glomerulonephritis (RPGN) is a rare presentation.[1] Renal involvement is usually secondary to the disease or to its treatment. Membranous glomerulonephritis is the most common form but there are other forms like secondary amyloidosis, mesangial glomerulonephritis, necrotizing glomerulonephritis, analgesic nephropathy or thin basement membrane disease.[2,3]

Clinical Case: A 54-year-old woman with a past diagnosis of positive anti-cyclic citrullinated peptide erosive RA under daily low dose prednisolone and 20 mg of methotrexate weekly presented with fever, weight loss, dry cough, hemoptysis and dyspnea. On physical examination, she appeared pale, normotensive, with a temperature of 37,4ºC. Bilateral basal biphasic rales were noted on pulmonary auscultation.

Laboratory values showed hypochromic microcytic anaemia (9,6 g/dL), elevated erythrocyte sedimentation rate (ESR) 62 mm/h, C-reactive protein 29,6 mg/dL and serum creatinine of 15 mg/L. Urinalysis showed a proteinuria of 1,3g/d with creatinine clearance of 81 mL/min; the sediment contained hyaline-granular casts. Perinuclear antineutrophil cytoplasmatic antibody (pANCA) were positive (198 UI/mL) with myeloperoxidase antineutrophil cytoplasmatic antibody (MPO-ANCA) specificity. Renal biopsy revealed a small-vessel vasculitis compatible with MPA.

Computerized tomography pulmonary scan showed diffuse parenchymal infiltrates secondary to pulmonary alveolar capillaritis and hemorrhage. Bronchoalveolar lavage showed neutrophilic alveolitis with positive hemossiderin in macrophages.

The diagnosis of MPA with renal and pulmonary involvement was made. Prompt treatment with 3 day pulses of 1 g/day of methylprednisolone and intravenous cyclophosphamide had an excellent response.

Conclusion: Vasculitis is not an uncommon extra-articular manifestation of RA. GNRP is a rare presentation, usually drug related- D-penicillamine (negative ANCA), bucillamine (negative ANCA), gold salts (positive ANA); in the context of secondary amyloidosis or in patients without disease-modifying antirheumatics drugs.[4,5,6-8,9]

MPA is a necrotizing pauci-immune vasculitis of small vessels without clinical or pathological evidence of granulomatous inflammation. ANCA positivity is found in most cases (25%-cANCA and 60% pANCA).[10]

The diagnosis of MPA must be kept in mind when a previously diagnosed RA patient presents with constitutional, renal or pulmonary symptoms/signs because early treatment is needed.

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P45 – R-Spondin1 Protects Against Inflammatory Joint Damage During Murine Arthritis by Modulating The Wnt Pathway

Abdollahi-Roodsaz S.,[1] Abo A.,[2] Akhemtshina A.,[3] Böhm C.,[3] Binnerts C.,[2] Bright J.M.,[2] David J.P.,[3] Distler J.,[3] Joosten L.,[1,4] Katzenbeisser J.,[3] Kim K.A.,[2] Kroenke G.,[3] Luther J.,[3] Schett G.,[3] Scholtysek C.,[3] Stock M.,[3] Surmann-Schmitt C.,[5] Tran C.,[2] Uderhardt S.,[3] Zaiss M.[3]

1. Dept. of Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
2. Nuvelo Inc., San Carlos, USA
3. Dept. of Internal Medicine 3 and Institute for Clinical Immunology
4. Dept. of Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
5. Dept. Experimental Medicine, University of Erlangen-Nuremberg, Germany

During the course of different musculoskeletal diseases joints are progressively damaged by inflammatory, infectious or mechanical stressors leading to joint destruction and disability.

While effective strategies to inhibit joint inflammation have been developed during the last deca-

de - particularly leading to development of targeted cytokine blocking therapy - the molecular mechanisms of joint damage are still poorly understood. Here, we show that the secreted Wnt pathway modulator R-Spondin-1 (RSpo1) is highly effective in preserving structural integrity of joints in a TNFα transgenic mouse model of arthritis by protecting bone and cartilage from inflammatory damage. RSpo1 antagonizes the Wnt inhibitor Dkk1 and modulates Wnt signalling in mesenchymal cells. In osteoblasts, RSpo1 induces differentiation and expression of OPG thereby inhibiting osteoclastogenesis *in vitro*. *In vivo*, RSpo1 promotes osteoblast differentiation and bone formation, blocks osteoclast development and globally modulates the homeostasis of anabolic and catabolic gene expression. We observed induction of genes involved in both chondrogenesis and osteogenesis, which contribute to integrity of cartilage and bone during joint inflammation.

Our results demonstrate the therapeutic potential of RSpo1 as an anabolic agent for the preservation of joint architecture.

P46 – Identification of a New Serological Marker for Chronic Autoimmune Pancreatitis

Simone R.,[1] Dolcino M.,[2] Frulloni L.,[3] Lunardi C.,[1] Corrocher R.,[1] Puccetti A.[2]

1. Dept. of Clinical and Experimental Medicine, Section of Internal Medicine, University of Verona, Verona
2. Giannina Gaslini Institute, Genova
3. Dept. of Biomedical and Surgical Sciences, University of Verona, Verona, Italy

Introduction: Autoimmune pancreatitis (AIP) is characterized by an inflammatory process in which prominent lymphocyte infiltration with associated fibrosis of the pancreas leads to organ dysfunction. The cause of the disease is still unknown. The autoimmune origin for AIP has been suggested but never proven and little is known about its pathogenesis.[1-3]

Aim: To identify pathogenetically relevant autoantigen targets specifically recognized by serum immunoglobulins of patients with autoimmune pancreatitis.

Patients and Methods: We screened a random peptide library with pooled IgG immunoglobulins obtained from 20 patients with AIP. Peptide specific immunoglobulins were affinity purified from the individual sera of patients with peptide-sepharose columns.[4]

Results: Among the identified peptides, one was recognized by the majority of patients' sera, but not by sera of normal donors and of patients with other autoimmune diseases. The peptide showed homology with a Helicobacter pylori derived protein and with UBR2, a protein ubiquitination enzyme highly expressed in acinar cells of the pancreas and in the kidney. Anti-peptide antibodies affinity purified from patients' sera recognized the Helicobacter derived protein and UBR2. Moreover antibodies against the bacterial epitope can be detected virtually in all the patients with AIP. Such reactivity was not detected in healthy controls and in patients with other pancreatic diseases, such as pancreatic adenocarcinoma, intraductal papillarymucinous neoplasm and alcoholic chronic pancreatitis.

Conclusion: Our findings suggest that Helicobacter pilori infection can be linked to the pathogenesis of AIP and that UBR2 can be considered a novel autoantigen target in AIP. Most importantly the identified peptide is able to discriminate AIP from other pancreatic disorders, particularly from pancreatic adenocarcinoma.

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P47 – B Cell Activating Factor Expression in Patients with Rheumatoid Arthritis – Effect of B Cell Depletion Therapy

Moura R.A.,[1] De La Torre Ortega I.,[2] Leandro M.J.,[2] Edwards J.C.W.,[2] Cambridge G.[2]

1. Instituto de Medicina Molecular, Rheumatology Research Unit, Lisbon, Portugal
2. University College London, Center for Rheumatology, London, UK

Introduction: The cytokine B-cell activating factor

(BAFF) is important for the maturation of B cells from the transitional stage onwards. Although defects in BAFF receptor expression do not necessarily result in immunodeficiency, follicular, marginal zone and class-switched memory B cells are very reduced in number and serum levels of IgM and IgG consequently low. B cell depletion therapy (BCDT) based on rituximab is now an established and effective therapy for RA. B cell return, predominantly with naïve B cells, invariably precedes relapse.

However in some patients there is a reduced capacity of returning B cells to undergo full maturation. In some, this results in a serious reduction in circulating IgG.

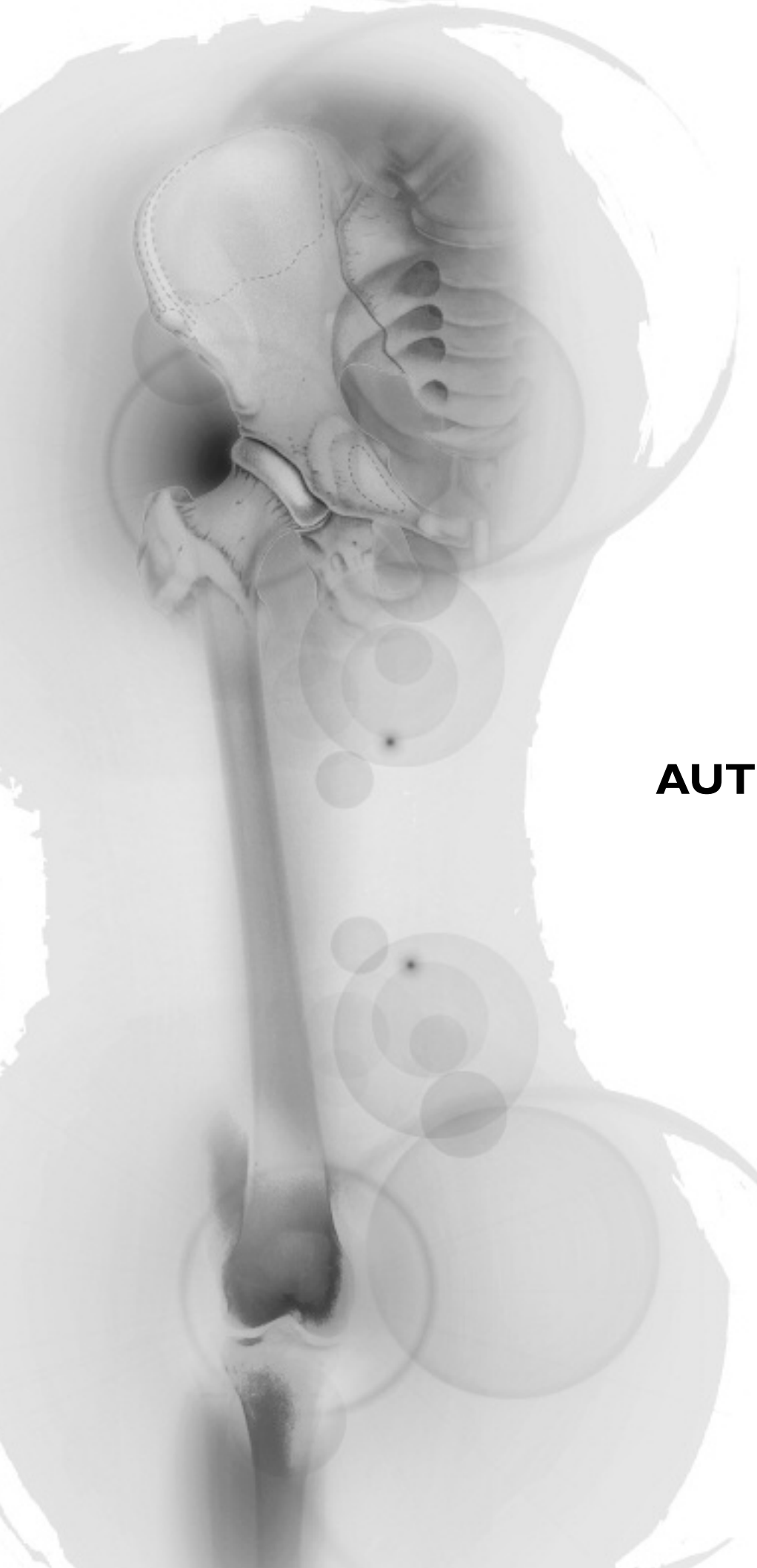
Aim: To examine the expression of BAFF-R on naïve (CD19+CD27-) and memory (CD19+CD27+) B cells in normal individuals and RA patients pre-BCDT and at B cell return.

Patients & Methods: Phenotypic analysis of peripheral blood mononuclear cells from normal controls (NC; n=9) and patients with RA (9 pre-BCDT and 12 at B cell return) were performed by flow cytometry using combinations of CD19, CD27 and anti-BAFF-R.

Results: There was no significant difference between the % naïve B-cells positive for BAFF-R in NC and patients pre-BCDT or at B cell return. The % of memory B-cells positive for BAFF-R was however significantly lower at B cell return compared with NC (69.2±19.6 vs 87.7±6.7%; p=0.02). When the mean fluorescent intensity (MFI) of BAFF-R binding was compared, patient memory B cells expressed significantly lower densities of receptors both pre-BCDT (55.7±14%; p=0.01) and at B-cell return (54.2±13.9%; p=0.005) compared with NC (79.4±19.3%). In CD27- populations, there was a significant difference between MFIs of NC (92.5±25.8%) and patients at B cell return (61.3±18.1%; p=0.005) but not compared with values obtained pre-BCDT (71.1±26%).

Conclusion: These preliminary studies show that BAFF-R expression is reduced on naïve CD27- B-cells pre-BCDT only and on CD27+ B-cells both pre-BCDT and at B cell return.

This suggests that patients with RA have a tendency towards low BAFF-R expression and this may increase the risk of low IgG developing in some patients following BCDT.



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